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WARSZAWSKA DRUKARNIA NAUKOWA — WARSZAWA, ŚNIADECKICH 8

Orientacyjne badania mikoflory najmłodszych części systemu korzeniowego topól (*Populus euramericana marilandica* Bosc.) z różnych stanowisk w Turwi

Analyse mycologique des radicelles et de jeunes racines du peuplier (Populus euramericana marilandica Bosc.) de divers milieux à Turew

W. TRUSZKOWSKA

Przedstawione opracowanie wiąże się, ze względu na zastosowaną metodę badawczą, z podobną pracą dotyczącą świerka (Mańka i Truszkowska 1958), która naświetla zagadnienie kontaktów jego korzeni z grzybami. Wyizolowane z poszczególnych części systemu korzeniowego świerka grzyby zostały potraktowane jako specyficzna grupa biologiczna, której skład gatunkowy może mieć bezpośredni wpływ na zdrowotność korzeni oraz związek z rozwojem drzew.

Tego rodzaju potraktowanie zagadnienia stało się bodźcem do podobnego przeanalizowania najmłodszych korzeni topól oraz gleby z najbliższego ich sąsiedztwa, z czterech różnych stanowisk w Turwi, gdzie drzewa wykazywały duże różnice przyrostu na wysokość i grubość.

Wspomniane topole, mniej więcej tego samego wieku, rosły w różnych warunkach siedliskowych. W okresie prowadzonych obserwacji miały około 20 lat. Wysokość drzew wahała się w granicach od 8,3 do 19 m, a pierśnica od 9 do 43 cm. Opracowanie wspomnianych stanowisk przedstawił Wilusz (1953). Ponadto przebadana była dynamika rozwojowa mikoryz danych drzew podczas rocznego okresu czasu (Truszkowska i Narkiewicz-Jodko 1955) oraz grzyby zasiedlające martwe i zamierające gałęzie (Truszkowska 1957). Dalszym ciągiem badań jest analiza mikologiczna zdrowych, najmłodszych korzeni i gleby z najbliższego ich sąsiedztwa dla uzupełnienia obserwacji.

MATERIAL I METODY BADAŃ

Materiał. Przedmiotem badań były najmłodsze części systemu korzeniowego drzew, to znaczy korzonki o budowie pierwotnej, przeważ-

nie przekształcone w mikoryzy oraz korzenie starsze, do 3 mm ϕ , wykazujące wtórny przyrost na grubość i gleba z najbliższego ich sąsiedztwa.

Materiał korzeniowy do badań pochodził tylko z warstwy próchniczej gleby. Na każdym stanowisku pobierano korzenie z trzech sąsiadujących ze sobą drzew, najlepiej charakteryzujących daną plantację. Równocześnie z materiałem korzeniowym pobierano próbki gleby spod każdego drzewa.

Sposób pobierania materiału i dalszego z nim postępowania był analogiczny jak w przypadku świerka (Mańka i Truszkowska 1958).

Metody badań. Metody badań laboratoryjnych były w zasadzie identyczne jak w odniesieniu do świerka, dlatego w aktualnej pracy zostaną omówione jedynie różnice w postępowaniu, odnoszące się do materiału topolowego.

Badania przeprowadzono metodą czystych kultur, która miała na celu ustalenie, choćby częściowo, składu gatunkowego grzybów wchodzących w kontakty z korzeniami drzew rosnących na obserwowanych stanowiskach.

Do tego celu posługiwano się szeregiem pożywek. Przede wszystkim wykorzystano podłoża opisane w pracy na temat świerka. Ponadto zastosowano pożywkę Warcupa (Warcup 1950), ziemniaczaną zwykłą i zakwaszoną, ryżową oraz ziemniaka w postaci skrawków wg przepisu Railly (1950), pożywkę agarową z mączki kukurydzianej bez peptonu (Ainsworth i Bisby, str. 213, 1954), pożywkę agarową Blakeslee stosowaną dla rodzaju *Mucor* (Rawlins, str. 79, 1933) z dodatkiem 25 γ biotyny na litr, pożywkę fasolową (Thurston, str. 186, 1957) oraz pożywkę stosowaną dla grzybów rozkładających drewno (Piefer, Humprey, Acree wg Rawlinsa, str. 81, 1933) z dodatkiem 25 γ witaminu B₁ na litr.

Przy izolacji grzybów z gleby zastosowano dodatkowo podłoże Warcupa.

Do oznaczania gatunków z rodzaju *Fusarium* korzystano z kompletu pożywek Railly. Pozostałe podłoża miały na celu sprowokowanie owocowania lub osiągnięcie bujniejszego wzrostu grzybní. Pierwszy cel osiągnięto w niewielkim stopniu dzięki pożywce fasolowej, drugi dzięki pożywce z mączki kukurydzianej oraz Blakesleego z biotyną i Piefera, Humpreya i Acreego z witaminem B₁. Pożywka kombinowana agarowo-wiórkowa nie dała tym razem efektów w postaci owocowania.

W związku z izolacją grzybów z korzeni topoli zastosowano nieco inny niż w przypadku świerka czas odkażania poszczególnych części materiału, a mianowicie: 50% alkoholem — mikoryzy 10 sek, korzenie od 1/2 do 3 mm ϕ 20 sek, roztworem sublimatu: mikoryzy 20 sek, korzenie do 1/2 mm ϕ 30 sek, do 1 mm ϕ 35 sek, a do 3 mm ϕ 40 sek. Przy izolacji

grzybów z gleby posłużono się dodatkowo metodą mieszania odrobiny rozpylonej gleby (5—20 mg) z przestudzoną, ale pozostającą w stanie płynnym pożywką.

WYNIKI BADAŃ WŁASNYCH

Zestawienie wyników badań przedstawiają kolejne tabele. Opisy oznaczonych grzybów umieszczono w tekście, w porządku alfabetycznym. Pominęto szczegółowe opisy tych kultur grzybów, które odpowiadały opisom zamieszczonym w badaniach dotyczących świerka.

Tabela 1

Wykaz izolacji wykonanych z mikoryz i korzeni topoli

Rodzaj materiału	Suma wykonanych izolacji	Liczba wykonanych izolacji na poszczególnych pożywkach			Suma
		gz*	W*	Wpz*	
Mikoryzy	216	72	72	72	864
Korzenie 1/2 mm Ø	216	72	72	72	
Korzenie 1 mm Ø	216	72	72	72	
Korzenie 1 — 3 mm Ø	216	72	72	72	

* gz — pożywka glukozowo-ziemniaczana, W — pożywka Waksmana, Wpz — pożywka Warcupa zmodyfikowana (Mańka i Truszkowska 1958).

W tabeli 1 przedstawiono ogólną ilość wykonanych izolacji z systemu korzeniowego topoli. W tabeli 2 zestawiono ogólną ilość otrzymanych kolonii grzybów.

Tabela 2

Ilość otrzymanych kolonii grzybów pochodzących z mikoryz i korzeni topoli

Rodzaj materiału	Suma otrzymanych wyosobnień	Liczba otrzymanych grzybów na poszczególnych pożywkach		
		gz	W	Wpz
Mikoryzy	154	51	53	50
Korzenie 1/2 mm Ø	166	61	59	46
Korzenie 1 mm Ø	177	55	65	57
Korzenie 1 — 3 mm Ø	172	48	71	53
Suma	669	215	248	206

Różnica pomiędzy ilością wykonanych izolacji a ilością otrzymanych wyosobnień grzybów przypada na kolonie bakterii, promienniczaków lub takie przypadki, kiedy z inokulów nic nie wyrosło.

Tabela 3

Wykaz izolacji wykonanych z gleby

Rodzaj materiału	Suma wykonanych izolacji	Liczba wykonanych izolacji na poszczególnych pożywkach			
		gz	W	Wpz	Wp*
Gleba 1	13	4	3	3	3
Gleba 2	13	4	3	3	3
Gleba 3	13	4	3	3	3
Gleba 4	13	4	3	3	3

*Wp — pożywka Warcupa

Tabele 3 i 4 zawierają takie same zestawienia dotyczące izolacji wykonanych z gleby. Z zestawień tych wynika, że najwięcej kolonii grzybów osiągnięto dzięki pożywce glukozowo-ziemniaczanej.

Tabela 4

Ilość otrzymanych kolonii grzybów z gleby

Rodzaj materiału	Suma kolonii	Liczba otrzymanych kolonii z gleby na poszczególnych pożywkach			
		gz	W	Wpz	Wp
Gleba 1*	96	45	18	21	12
Gleba 2	53	12	16	8	17
Gleba 3	52	23	9	10	10
Gleba 4	67	33	9	10	15
Suma	268	113	52	49	54

*Gleba 1 — stanowisko 1 na śródleśnej polanie, Leśnictwo Turew.

Gleba 2 — stanowisko 2 na glebie torfowej Leśnictwo Turew.

Gleba 3 — stanowisko 3 na glebie porolnej w Ćwikłowie.

Gleba 4 — stanowisko 4 dwurzędowa aleja topolowa w okolicy Turwi.

ZESTAWIENIE OKREŚLONYCH GRZYBÓW

1. *Absidia sphaerosporangioides* Mańka i Truszkowska — Wymiary trzonków zarodnionośnych $131,1-241,5 \times 6,1-7,9 \mu$, kolumella $13,8-25,3 \times 16,1-18,4 (23) \mu$, zarodniki zarodniowe, okrągłe, $2,8-3,3 \mu$, zarodnie, kuliste, $21,6-32,4 \mu$.

2. *Botrytis cinerea* Persoon — Konidia $8,8-12,5 \times 7,5-10 \mu$. Tylko z pierwszego odszczepienia wyrosło owocowanie konidialne,

ze wszystkich następnych bez względu na podłoże wyrastała tylko grzyb-
nia i skleroty.

3. *Cephalosporium acremonium* Corda — Konidiofory $46-87,4 \times 2-2,3 \mu$, konidia $4,6-7,4 (9,2) \times 1,5-2,3 \mu$.

4. *Cephalosporium asperum* March. — Konidia $4,6-5,6 \times 3,3-3,8 \mu$.

5. *Chaetomium indicum* Corda — Poszczególne cechy morfologiczne zgodne z opisem Munka (1957), których nie przytacza się ze względu na pospolitość gatunku.

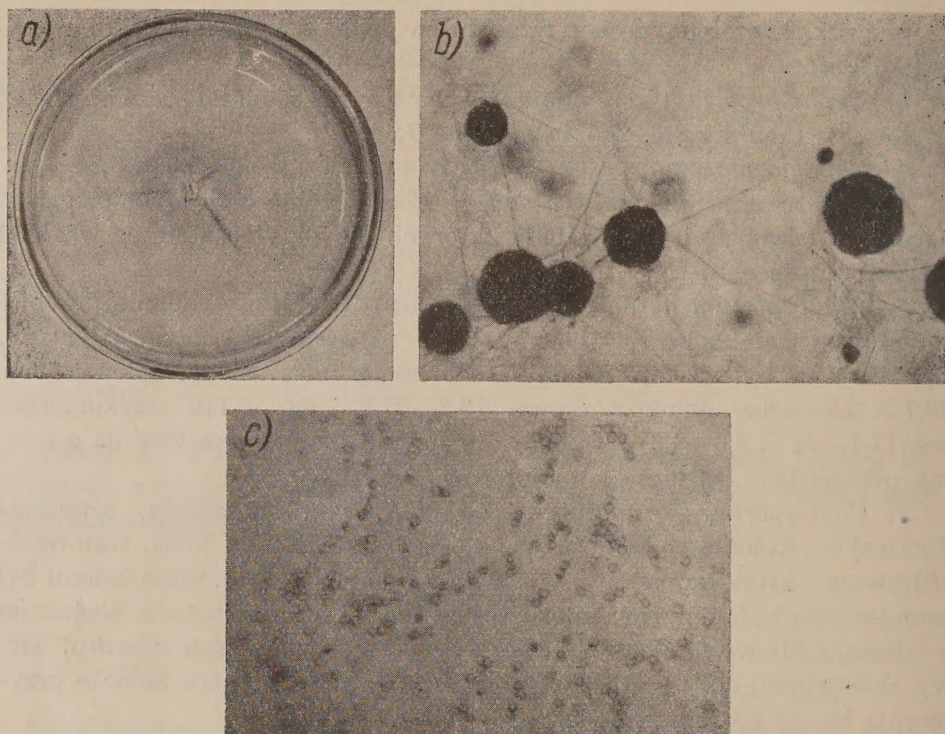
6. *Cladosporium herbarum* (Persoon) Link. — Kolonie przybierały formę ciemnooliwkowych murawek z szarawymi przejaśnieniami. Spód kolonii był czarny. Konidia 1—3 komórkowe: niepodzielone, $4,6-20,7 \times 2,8-4,6 \mu$, dwukomórkowe, $13,8-27,6 \times 4,6-5,1 \mu$, trzykomórkowe, $17,1-23 \times 3,8-4,6 \mu$. Trafiały się również pojedyncze konidia z trzema przegrodami, grubość konidioforów, $5,6-9,2 \mu$.

7. *Coniosporium arundinis* (Corda), [syn. *Popularia arundinis* Fries] — Kolonia rosła dość szybko, początkowo była biała, watowato-filcowata z kropelkami żółtej wydzieliny na powierzchni. Spód kolonii był promieniście pofałdowany koloru żółtawego do szarożółtego, z naciekami o odcieniu liliowym. Konidia tworzyły się soczewkowatego kształtu, barwy złoto-brunatnej, $9,2-12,5 \mu \varnothing$, $5,6-7,2 \mu$ grubości. Stare kolonie przybierają barwę czarnobrunatną.

8. *Coniosporium arundinis* Sacc., [syn. *Papularia sphaerosperma* (Persoon) v. Höhnelt] — Kolonie były początkowo białe, później intensywnie żółte, o niskiej grzybni powietrznej, lekko sfilcowanej. Spód kolonii był brązowy. Owocowanie konidialne tworzyło niekiedy skupienia, widoczne nawet gołym okiem, w postaci ciemnych punktów. Konidia miały kształt soczewkowaty, charakteryzował je gruby, silnie łamiący światło pierścień dookoła. Barwy były złoto-brunatnej, tworzyły się jak owoce na zakończeniach krótko rozgałęzionych trzonek konidialnych, wymiary konidiów $5,1-6,9 \mu \varnothing$, $3,1-4,6 \mu$ grubości.

Gatunek *Coniosporium arundinis* został opisany dwukrotnie wg dwu różnych autorów, ponieważ stwierdzono dużą różnicę w wymiarach konidiów. Zachodzi wobec tego pytanie, czy mogą to być dwa różne gatunki, czy też jeden o bardzo dużej zmienności. Ponadto zasadniczą trudność przedstawia ustalenie nazwy rodzajowej. Czy należy przyjąć nazwę *Coniosporium*, czy *Papularia*. U starszych autorów (Lindau 1907) figuruje rodzaj *Coniosporium*, a jego synonimem jest *Papularia*. W nowej literaturze Barnett (1956) podaje tylko rodzaj *Coniosporium*, a nie uwzględnia *Papularia*. W literaturze angielskiej figuruje przede wszystkim rodzaj *Papularia*, a jako synonim *Coniosporium* (Gilman 1959, Ainsworth i Bisby 1954).

9. *Coniothyrium bohmani* sp.n. — Na powierzchni kolonii widoczna była niska, biała, zbijająca się w kłaczki, powietrzna grzybnia. Spód kolonii stanowiła cienka warstewka czarnobrunatnej, zbitej pлектенchy-my. Bardzo drobne, czarne piknidy tworzyły się licznie wewnątrz po-



Ryc. 1. *Coniothyrium bohmani* sp. n. a — kolonia na pożywce maltozowej; b — fragment kolonii przedstawiający kuliste piknidy; c — konidia.

żywki lub na jej powierzchni w przypadkach, kiedy zbity plektenchyma nie zakładała się na całej przestrzeni podłoża odżywczego. Grzybnia powietrzna tworzyła się biała, hialinowa z licznymi kropelkami tłuszczu wewnątrz strzępek o grubości $2,3-4,6\mu$, piknidy kuliste, czarne z otworem na szczycie, $95-184\mu$, konidia kuliste, w masie barwy brunatnej, pod mikroskopem oliwkowe, $2,8-3,7 (4,6)\mu$ $\varnothing$ przeważnie z jedną kropelką tłuszczu wewnątrz (wykonano 50 pomiarów), ryc. 1, 1a, 1b.

Coniothyrium bohmani sp. n. — *Pycnidia subsuperficialia vel superficialia, sparsa, gregaria, atra, globosa et pertusa*, $95-184\mu$ $\varnothing$. *Sporulis glöbosis, olivaceis, 1 guttulatis*, $2,8-3,7 (4,6)\mu$ $\varnothing$.

10. *Coniothyrium fuckelii* Sacc. — Kolonie były niskie, barwy szarej lub piaskowoszarej, filcowate z dużymi, czarnymi piknidami na powierzchni oraz kropkami bladożółtymi wydzielin. Spód kolonii był barwy pożywki, piknidy kuliste z brodawkowato wykształconym ujściem, $200-320\mu$ $\varnothing$, konidia oliwkowej barwy, jajowate, do prawie cylindrycznych, $2,8-3,3 \times 3,3-4,6\mu$.

11. *Cylindrocarpon didymum* (Hartung) Wollenweber [syn.

Ramularia eudidyma Wollenweber] — Kolonia początkowo biała, później piaskowożółta, niska, rosła powoli. Spód kolonii był barwy ciemnej ochry, konidia 1—4-komórkowe: jednokomórkowe, $9,2—11,5 \times 3,5—5,1 \mu$, dwukomórkowe, $20,7—27,6 \times 4,6—5,1 \mu$, czterekomórkowe, $34,5—39,1 \times 5,6—6,1 \mu$.

12. *Cylindrocarpon radicola* Wollenweb. — [*Ramularia macrospora* Wollenweb.] — Konidia 1—4-komórkowe: jednokomórkowe, $11,5—16,1 \times 3,3—5,1 \mu$, dwukomórkowe, $25,3—29,9 \times 4,6—6,1 \mu$, czterekomórkowe, $32,2—39,1 \times 5,6—6,9 \mu$.

13. *Fusarium solani* (Mart.) App. et Wr. f. 3 nov. (Raiłło 1950) — Na zwykłej pożywce ziemniaczanej grzybnia powietrzna była biała, dość nikła, lekko sfilcowana. Na ryżu wystąpił barwik kremowo-żółty aż do barwy ochry. Mikrokonidia tworzyły się w główkowatych skupieniach.

Mikrokonidia bez przegród, $6,9—11,5 \times 2,8—3,8 \mu$

„ z 1 przegrodą, $16,1—20,7 \times 3,8—4,6 \mu$

Makrokonidia z 3 przegrodami, $36,8—39,1 \times 5,1—5,6 \mu$

„ z 5 przegrodami, $41,4—64,4 \times 5,6—6,4 \mu$

Chlamydospory kuliste, $11,5—13,8 \mu \emptyset$.

Wszystkie *Fusaria* oznaczono wg klucza Raiłły (1950) na standardowych pożywkach.

14. *Fusarium martii* App. et Wr. 1 comb. nov. (Raiłło 1950) — Na zwykłej pożywce ziemniaczanej kolonia była biała, dość puszysta, lekko zbijająca się w kłaczki. Na ryżu wystąpił barwik kremowożółty dookoła przestrzeni pomiędzy ziarnami.

Mikrokonidia bez przegród, $6,9—16,1 \times 3,3—4,6 \mu$

„ z 1 przegrodą, $18,4—25,3 \times 4,6—5,6 \mu$

Makrokonidia z 3 przegrodami, $32,2—41,4 \times 4,8—5,6 \mu$

Chlamydospory były pojedyncze, $6,9—9,2 \times 9,2—11,5 \mu$ lub podwójne, $11,5—18,4 \times 9,2—11,5 \mu$, śródstrzepkowe lub końcowe.

15. *Fusarium trichothecioides* Wr. — Na zwykłej pożywce ziemniaczanej kolonia rosła biała, niska, o skąpej grzybni powietrznej. Na ryżu wystąpił barwik żółty, miejscami z różowym odcieniem.

Mikrokonidia bez przegród, $7—9,2 \times 2,3—3,3 \mu$

„ z 1 przegrodą, $20,7—27,6 \times 6,9—7,4 \mu$

„ z 2 przegrodami, $23—30 \times 6,9—7,4 \mu$

Makrokonidia z 5 przegrodami, $31,2—32,2 \times 6,9—7,4 \mu$.

16. *Fusarium sporotrichioides* Sherb. subsp. minus (Wr.) comb. nov. (Raiłło 1950) — Na pożywce zwykłej ziemniaczanej kolonia była biała, włóknista, przylegająca do podłoża, o skąpej grzybni powietrznej. Na ryżu wystąpił barwik żółty, gdzieś żółtooliwkowy, miejscami

z brunatnymi naciekami. Mikrokonidia tworzyły się dwu rodzajów: cylindryczne i gruszkowate lub cytrynkowate.

- Mikrokonidia bez przegród, cylindryczne, $9,2-13,8 (16,1) \times 3,8-4,6 \mu$
 „ bez przegród, gruszkowate lub cytrynkowate, $9,2-18,4 \times 6,9-9,2 \mu$
 „ z 1 przegrodą, cylindryczne, $14,8-20,7 \times 3,8-5,1 \mu$
 „ z 1 przegrodą gruszkowate lub cytrynkowate, $13,8-20,7 \times 6,9-8,4 \mu$

Makrokonidia z 3 przegrodami, $23-34,5 \times 4,6-5,1 \mu$.

Raillo nie wspomina o mikrokonidiach cytrynkowatych i gruszkowatych, które opisuje Wollenweber i Reinking (1936).

17. *Fusarium conglutinans* Wr. var. *betae* Stewart — Na zwykłej pożywce ziemniaczanej wyrosły kolonie białe, watowate, delikatne. Na ryżu występował barwik żyworóżowy, przechodzący w czerwień z odcieniem fioleto.

- Makrokonidia z 3 przegrodami, $39,1-48,3 \times 3,8-4,6 \mu$
 „ z 5 przegrodami, $48,3-52,9 \times 3,8-4,6 \mu$.

Chlamydospery, z brodawkami na powierzchni, występowały licznie, przeważnie na końcach strzępek, pojedynczo lub w łańcuszkach, jedno- lub dwukomórkowe. Wymiary jednokomórkowych, okrągłych, $10,2-11,5 \mu$, owalnych, $12,5-13,8 \times 9,2-11,5 \mu$, dwukomórkowych, owalnych, $18,4-23 \times 11,5-14,3 \mu$.

18. *Fusarium semitectum* Berk. et Rav. — Na pożywce ziemniaczanej, zwykłej kolonia wyrosła biała, puszysta. Na ryżu wystąpił barwik żółtobrunatny.

- Mikrokonidia bez przegród, $6,9-13,8 \times 2,8-3,8 \mu$
 „ z 1 przegrodą, $16,1-23 \times 3,8-4,6 \mu$
 Makrokonidia z 3 przegrodami, $25,3-30 \times 3,8-4,6 \mu$
 „ z 5 przegrodami, $25,3-32 \times 3,8-4,6 \mu$.

Chlamydospery znajdowano śródstrzępkowe, w krótkich łańcuszkach, przeważnie pojedyncze, okrągłe, $11,5-23 \mu$.

19. *Fusidium viride* Grove — Kolonia biała, niska, aksamitna, rosła dość powoli. Spód kolonii był barwy pożywki. Grzybnia tworzyła się hialinowa, delikatna o średnicy strzępek $1,5-2,5 \mu$. Konidia były wrzecionowatego kształtu, niekiedy ostro zakończone, proste lub lekko zgięte, wyrastały na zakończeniach prostych, pojedynczych trzonków konidialnych, wymiary $9,2-11,5 (13,8) \times 2,3-3,3 \mu$.

20. *Gliocladium deliquescens* Sopp. — Kolonie najlepiej rosły na pożywce maltozowej, na pożywce Czapek-Doxa słabo lub wcale. Na pożywce maltozowej wyrastała biała niska grzybnia powietrzna zbijająca się w drobne kłaczki. Na powierzchni grzybni tworzyło się, widoczne gołym okiem, owocowanie konidialne w postaci drobnutkich, czarnych

kropelek. Spód kolonii był barwy pomarańczowej. Pędzelki tworzyły się dwupiętrowe, konidia sklejały się w główki śluzowatą wydzieliną, średnica główek 11,5—23 μ , konidiów 7,9—9,2 $\times$ 2,3—2,8 μ .

21. *Hormodendrum olivaceum* Bonorden — Ciemnooliwkowa, aksamitna kolonia, promieniście bruzdowana, owocowała na całej powierzchni. Spód kolonii był czarny, trzonki konidialne 64,8—132,6 $\times$ 2,3—3,8 μ , drzewkowato rozgałęzione, konidia tworzyły się w krótkich łańcuszkach, 9,2—12,5 $\times$ 3,8—5,6 μ .

22. *Hormodendrum resinae* Lindau — Kolonie wyrosły w formie gęstych, aksamitnych murawek brunatnozielonej barwy. Trzonki konidialne rozgałęziały się drzewkowato na szczycie, konidia były jednokomórkowe, 5,6—6,9 $\times$ 3,3—4,6 μ .

23. *Hyalopus tener* Preuss — Biała aksamitna kolonia rosła powoli, spód był barwy pożywki, gdzieś z naciekami lilioworóżowymi. Konidia tworzyły zlepione śluzem główki o średnicy 4,3—6,3 μ , trzonki konidialne 8—25,3 $\times$ 2,1—2,6 μ , konidia cylindryczne, zaokrąglone na końcach, hialinowe, 3,1—5,2 $\times$ 2,3—3,6 μ .

24. *Monotospora daleae* Mason — Kolonie, początkowo barwy kremowej, ze skąpą, watowatą grzybnią powietrzną, widoczną tylko w części środkowej, z biegiem czasu przybierały barwę szarobrunatną, czemu towarzyszyło tworzenie się promienistych fałd. Spód kolonii był szarobrunatny. Trzonki konidialne były krótkie, pojedyncze, konidia kuliste, barwy złotobrunatnej, o grubych błonach, a średnicy 11,2—15 μ .

25. *Mortierella pusilla* var. *isabellina* Oudemans — Kolonie niskie aksamitne, lekko strefowane, początkowo białe, później szarozielonawe rosły szybko. Spód był barwy pożywki. Zarodnie osiągały średnicę 16—27,6 (34,5) μ , zarodniki zarodniowe kuliste, 2,3—4,6 μ .

26. *Mortierella pusilla* var. *vinacea* Dixon-Steward — Średnica zarodni 10,8—16,1 (21,6) μ , zarodniki zarodniowe okrągłe, 2,3—2,8 μ , długość trzonek zarodniowych 120—184 μ .

27. *Mucor hiemalis* Wehmer — Średnica zarodni 43,2—64,8 μ , kolumella 27,6—46,7 $\times$ 23—43,7 μ , zarodniki zarodniowe 4,6—7,9 $\times$ 2,3—6,1 μ .

28. *Mucor ramannianus* Möller — Średnica zarodni 16—20,7 μ , zarodniki zarodniowe 2,3—3,3 μ , komórki olbrzymie 41,4—43,7 $\times$ 27,6—34,5 μ .

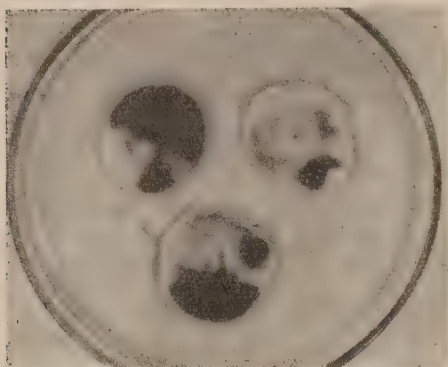
29. *Mycelium radices atrovirens* Melin — Średnica strzępek grzybni powietrznej 2,3—3,8 μ , a grzybni pożywkowej 3,3—6,2 $\times$ 3,8—4,6 μ .

30. *Penicillium commune* Thom — Wymiary gałęzi 16,1—23 $\times$ 2,8—3,3 μ , metul 6,1—14,8 $\times$ 2,8—3,8 μ , sterygm 7,4—11,5 $\times$ 2,3—2,8 μ , konidiów 3,8—5,1 μ .

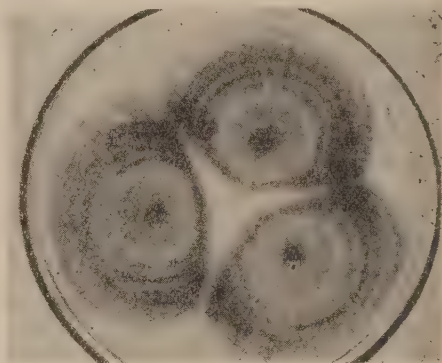
31. *Penicillium waksmani* Zaleski — W zaobserwowanym przypadku obraz kolonii odpowiadał opisowi 4 wariantu z poprzedniej pracy

(Mańska i Truszkowska 1958). Wymiary gałęzi 11,5—16,1 (41,4) $\times$ $\times$ 2,3—2,8 μ , sterygm 6,9—9,2 $\times$ 1,8—2,3 μ , konidów 2,3—2,8 μ (ryc. 2).

32. *Penicillium thomi* Zaleski — Po 12 dniach kolonie osiągnęły średnicę 3 cm, barwę szaro-niebiesko-zieloną (Y 10, G 10, B 20)* z białym pierzasto poszarpanym brzegiem. Na powierzchni kolonii tworzyły się bardzo liczne, różowe, twarde, kruche skleroty oraz obfite, bezbarwne



Ryc. 2. *Penicillium waksmani*. Kolonie na pożywce Czapek—Doxa



Ryc. 3. *Penicillium thomi*. Kolonie na pożywce Czapek—Doxa

wydzieliny. Zapach wydzielają kultury słaby i nieokreślony. Pędzelki były typowe dla *Monoverticillata*, czasem trafiały się rozgałęzione. Pędzelki tworzyły się zwarte na długim trzonku, którego długość bardzo trudno było ustalić od 253 μ począwszy, a średnicy 3,3—4,6 μ , o powierzchni delikatnie brodawkowanej. Sterygmy charakteryzował układ równoległy 9,2—11,5 $\times$ 2,3—2,8 μ , konidia eliptyczne lub prawie okrągłe 3,3—3,8 $\times$ 2,8—3,3 μ (ryc. 3).

33. *Penicillium lanoso-coeruleum* Thom — Kolonie po 12 dniach dorastają do 5 cm średnicy, barwy były w strefach brzeżnych niebiesko-zielonej (G 20, Y 30, B 30), a w środkowych szaro-niebiesko-zielonej (G 05, Y 50, B 40). Kolonie wykazywały promieniste bruzdowanie ze słabo lub wcale nie zaznaczonym strefowaniem koncentrycznym. Brzeg kolonii był biały do 2 mm szeroki. Niewielkie kropelki bezbarwnej wydzieliny obserwowano na skraju kolonii. Spód kolonii był na skraju bezbarwny, w środku kremowy. Woń słaba podobna do *Penicillium commune*. Trzonki konidialne były rozgałęzione, gałęzie 16,1—23 $\times$ 2,8—3,8 μ , metule 11,5—13,8 $\times$ 2,3—3,3 μ , sterygmy 9,2—10,7 $\times$ 2,3—3,8 μ , konidia eliptyczne do prawie okrągłych, 3,8—4,6 $\times$ 3,3—3,8 μ .

34. *Pestalozzia truncata* Lev. — Kolonie rosły białe, niskie, watowate, w okresie owocowania, w środku tworzyły się czarne wysepki.

* Barwy podano wg M. Locquin, 1957, *Chromotaxia*, Paris.

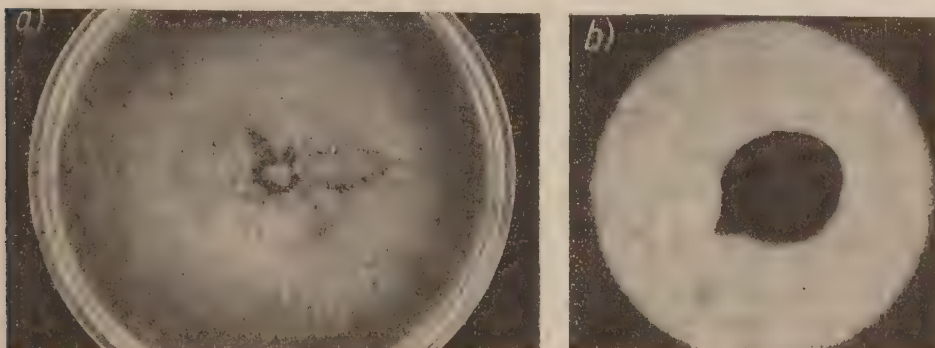
W tych miejscach tworzyły się łoża zarodnikonośne. Konidia $18,4\text{--}23 \times 6,9\text{--}9,2 \mu$, wykształcały od 2 do 4 witek na szczycie.

35. *Phoma humicola* Gilman et Abbott — Kolonie początkowo kremowej barwy z biegiem czasu brunatniały. Grzybnia powietrzna była bardzo niska, spód kolonii barwy pożywki. Piknidy tworzyły się przeważnie w środku kolonii, prawie kuliste, barwy brunatnej do czarnej, $230\text{--}322 \times 322\text{--}368 \mu$, konidia (7,9) $9,2\text{--}11,5 \times 3,3\text{--}4,6 \mu$.

36. *Phoma turviensis* sp. n. — Kolonie były barwy brunatnoszarej, watowatofilcowate, a spodzie czarnobrunatnym. Gęsto podzielona grzybnia powietrzna była barwy oliwkowozielonej, średnica strzępek $2,3\text{--}5,6 \mu$. Grzybnia tworzyła niekiedy śródstrzępkowe chlamydospory, kuliste, $9,2\text{--}11,5 \mu$. W momencie zasychania podłoża tworzyły się liczne drobniutkie, czarne piknidy. $122\text{--}255 \mu \phi$, z brodawkowato wykształconym ujściem, $23,5\text{--}34,5 \times 45 \mu$ szerokości u nasady. Konidia były eliptyczne lub jajowate, hialinowe, jasnozielonkawej barwy z 1 lub 2 kropelkami tłuszczu wewnątrz, $3,1\text{--}4,3 (5) \times 2,5\text{--}3,1 \mu$ (wykonano 50 pomiarów) (ryc. 4 i 4a).

Phoma turviensis sp. n. — *Pycnidia numerosa, superficialia vel depressa, atra cum ostiolo acute conoideo*, $122\text{--}255 \mu \phi$. *Sporulis oblongis vel ovoideis, hialinis 1 vel 2 guttulis*, $3,1\text{--}4,3 (5) \times 2,5\text{--}3,1 \mu$.

37. *Phoma* sp. — Na pożywce fasolowej wyrosły kolonie barwy kremowej, z żółtym strefowaniem. Grzybnia powietrzna sprawiała wrażenie



Ryc. 4. *Phoma turviensis* sp. n. a — kolonia na pożywce maltozowej; b — fragment kolonii przedstawiający pojedynczą piknidę

niziutkiego meszku, skąpego w strefach środkowych. Z biegiem czasu kolonie szarzały. Strzępki grzybni były barwy przydymionej, $1,5\text{--}4,6 \mu$ średnicy, piknidy barwy oliwkowej, kuliste, $34,5\text{--}50,6 \mu$, konidia cylindryczne lub jajowate, hialinowe, zielonkawe, $3,3\text{--}4,6 \times 2,3\text{--}2,8 \mu$. Ponieważ owocowanie wystąpiło tylko jeden raz nie udało się oznaczyć do gatunku.

Tabela 5

Ilościowy rozdział wyizolowanych grzybów na poszczególne kategorie badanego materiału

Lp.	Oznaczenie grzyba	Kategorie badanego materiału					Suma
		Miko- ryzy	korzenie o średnicy			gleba	
			do 1/2 mm	do 1 mm	do 3 mm		
1	<i>Absidia sphaerosporangioides</i>	7	11	7	6	—	31
2	<i>Botrytis cinerea</i>	6	—	—	—	—	6
3	<i>Cephalosporium acremonium</i>	12	6	—	—	—	18
4	„ <i>asperum</i>	1	—	1	1	—	3
5	<i>Chaetomium indicum</i>	—	4	—	—	—	4
6	<i>Cladosporium herbarum</i>	1	—	—	2	1	4
7	<i>Coniosporium arundinis</i>	—	—	—	1	6	7
8	<i>Coniothyrium bohmani</i>	—	—	—	—	5	5
9	„ <i>fuckelii</i>	1	—	—	—	—	1
10	<i>Cylindrocarpon didymum</i>	—	1	—	—	—	1
11	„ <i>radicicola</i>	—	1	1	1	—	3
12	<i>Fusarium solani</i> f.3.	35	34	75	19	1	164
13	„ <i>conglutinans</i> v. <i>betace</i>	—	—	—	2	9	11
14	„ <i>trichothecioides</i>	—	14	5	4	—	23
15	„ <i>martii</i> 1 comb.	—	1	4	2	2	9
16	„ <i>semitectum</i>	—	4	—	1	—	5
17	„ <i>sporotrichioides</i>	—	1	—	1	1	3
18	<i>Fusidium viride</i>	12	5	5	1	—	23
19	<i>Gliocladium deliquescens</i>	—	—	—	—	2	2
20	<i>Hormodendrum olivaceum</i>	2	1	—	4	—	7
21	„ <i>resinae</i>	12	5	5	1	—	23
22	<i>Hyalopus tener</i>	—	—	—	—	4	4
23	<i>Monotospora daleae</i>	—	—	—	—	1	1
24	<i>Mortierella pusilla</i> var. <i>isabellina</i>	1	—	—	—	—	1
25	<i>Mortierella pusilla</i> var. <i>vinacea</i>	—	1	—	—	2	3
26	<i>Mucor hiemalis</i>	—	—	—	—	1	1
27	„ <i>ramannianus</i>	—	—	—	—	1	1
28	<i>Mycelium radices atrovirens.</i>	2	1	10	2	1	16
29	<i>Penicillium commune</i>	—	2	1	4	4	11
30	„ <i>lanoso-coeruleum</i>	—	—	—	2	4	6
31	„ <i>thomi</i>	—	—	—	—	1	1
32	„ <i>waksmani</i>	2	—	—	8	27	37
33	<i>Pestalozzia truncata</i>	—	2	—	—	—	2
34	<i>Phoma humicola</i>	—	—	—	1	—	1
35	<i>Phoma turviensis</i>	1	—	—	—	—	1
36	<i>Phoma</i> sp.	—	—	—	—	1	1
37	<i>Phytophthora sim. cactorum</i>	—	—	—	—	2	2
38	„ sp. 1	—	—	—	—	35	35
39	„ sp. 2	—	—	—	—	12	12
40	<i>Plenodomus lingam</i>	—	2	5	—	—	7

c.d. tabl. 5

Lp.	Oznaczenie grzyba	Kategoria badanego materialu					Suma
		Miko- ryzy	korzenie o średnicy			gleba	
			do 1/2 mm	do 1 mm	do 3 mm		
41	<i>Pullularia pullulans</i>	1	1	—	1	—	3
42	<i>Septonema secedens</i>	2	—	—	—	—	2
43	<i>Spicaria griseola</i>	—	—	—	1	—	1
44	„ <i>simplicissima</i>	—	—	—	—	1	1
45	<i>Stemphylium piriforme</i>	2	2	1	—	2	7
46	<i>Tilachlidium humicola</i>	—	—	—	—	2	2
47	<i>Torula allii</i>	—	—	—	1	—	1
48	„ <i>lucifuga</i>	1	—	—	—	—	1
49	<i>Trichoderma album</i>	6	—	5	—	—	11
50	„ <i>glaucum</i>	1	3	—	31	13	48
51	„ <i>koningi</i>	11	11	20	55	61	158
52	„ <i>lignorum</i>	—	1	—	23	2	26
53	<i>Verticillium candidulum</i>	—	—	—	—	2	2
54	„ <i>terrestre</i>	2	—	—	—	2	4
55	<i>Zygorhynchus vuillemini</i>	—	—	—	—	54	54
56	Grzybnie nie owocujące	35	38	29	13	5	120
		156	152	174	188	267	937

33. *Phytophthora similis cactorum* Leb. et Cohn) Schr. — Delikatne białe, puszyste kolonie rosły dość szybko. Spód kolonii był barwy kremowej, strzępki grzybni powietrznej łatwo zbijały się w sznury, ponadto charakteryzowały je nieregularne rozdęcia na całej nieraz długości, co w efekcie dawało falistą linię brzegu. Średnica strzępek wahała się w dość dużych granicach 1,5—9,2 μ . Zarodnie pływkowe były przeważnie gruszkowatego czasem eliptycznego kształtu, 26—48,3 $\times$ 20—39,1 μ . Chlamydospory tworzyły się śródstrzępkowo, kuliste, 18,4—55,2 μ . Cospor nie zaobserwowano dlatego oznaczenie podano z zastrzeżeniem. Większość zaobserwowanych cech morfologicznych okazała się zgodna z opisem Frezzi (1950).

39. *Phytophthora* sp. 1 — Kolonie były podobne do wyżej opisanych (38), grzybnia powietrzna delikatna, biała, puszysta. Strzępki grube o falistej linii, miejscami bardzo obficie wypełnione kropelkami tłuszczu. gdzieniegdzie wytwarzały przegrody poprzeczne, średnica strzępek wahała się znowu w dużych granicach, 1,5—9,2 μ . Ponadto owocowania nie zaobserwowano na żadnej z zastosowanych pożywek. Obraz samej kolonii oraz grzybni wskazuje na przynależność kultury do rodzaju *Phytophthora*. co jednak trzeba traktować z zastrzeżeniem.



Ryc. 5. Turew, stanowisko 1, na śródleśnej polanie

40. *Phytophthora* sp. 2. — Kolonie znowu dosyć podobne do wyżej opisanych (38), ale obraz mikroskopowy wykazywał wyraźne różnice. Grzybnia tworzyła charakterystyczne rozdęcia, średnica strzępek $2,3-11,5\ \mu$, chlamydospory zaobserwowano kuliste, $18,4-23\ \mu$ oraz bardzo drobne zarodnie $9,2-11,5 \times 6,9-9,2\ \mu$. Znowu jest to przypadek, który jedynie z zastrzeżeniem można zaliczyć do rodzaju *Phytophthora*.

Tabela 6

Ilościowy rozdział grzybów wyhodowanych z poszczególnych kategorii badanego materiału z uwzględnieniem 4 różnych stanowisk

l.p.		Oznaczenie grzyba	Kategorie badanego materiału																				Suma
			mikoryzy				korzenie 1/2 mm				korzenie 1 mm				korzenie 1—3 mm				gleba				
			1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	
1	<i>Absidia sphaerosporangioides</i>	1	—	—	6	11	—	—	—	4	—	1	2	3	—	3	—	—	—	—	—	—	31
2	<i>Botrytis cinerea</i>	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	6
3	<i>Cephalosporium acremonium</i>	—	12	—	—	—	6	—	—	—	—	—	—	—	—	—	—	—	—	—	—	18	
4	„ <i>asperum</i>	—	—	—	1	—	—	—	—	—	—	1	—	1	—	—	—	—	—	—	—	3	
5	<i>Chaetomium indicum</i>	—	—	—	—	4	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	
6	<i>Cladosporium herbarum</i>	—	—	1	—	—	—	—	—	—	—	—	—	2	—	—	—	—	—	1	—	4	
7	<i>Coniosporium arundinis</i>	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	5	—	1	7	
8	<i>Coniothyrium bohdamii</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	5	—	—	5	
9	„ <i>fuckelii</i>	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
10	<i>Cylindrocarpon didymum</i>	—	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
11	„ <i>radicicola</i>	—	—	—	—	—	1	—	—	1	—	—	—	—	—	—	—	—	—	—	—	3	
12	<i>Fusarium solani</i> f. 3.	22	2	9	2	22	2	2	8	34	10	16	15	9	8	1	1	—	—	1	—	164	
13	„ <i>conglutinans</i> v. <i>betae</i>	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	9	—	—	11	
14	„ <i>trichothecioides</i>	—	—	—	—	4	10	—	—	—	5	—	—	—	4	—	—	—	—	—	—	23	
15	„ <i>martii</i> 1 comb.	—	—	—	—	1	—	—	—	4	—	—	—	—	—	—	2	—	1	—	1	9	
16	„ <i>semitectum</i>	—	—	—	—	—	1	2	1	—	—	—	—	—	1	—	—	—	—	—	—	5	
17	„ <i>sporotrichioides</i>	—	—	—	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	1	3	
18	<i>Fusidium viride</i>	7	1	3	1	1	1	2	1	5	—	—	—	—	1	—	—	—	—	—	—	23	
19	<i>Gliocladium deliquescens</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	2	
20	<i>Hormodendrum olivaceum</i>	—	—	2	—	—	—	—	1	—	—	—	—	—	—	—	4	—	—	—	—	7	
21	„ <i>resinae</i>	7	1	3	1	1	1	2	1	5	—	—	—	—	1	—	—	—	—	—	—	23	
22	<i>Hyalopous tener</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	4	4	
23	<i>Monotospora daleae</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	
24	<i>Mortierella pusilla</i> v. <i>isabellina</i>	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
25	„ v. <i>vinacea</i>	—	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	3	
26	<i>Mucor hiemalis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	1	
27	„ <i>ramannianus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	1	
28	<i>Mycelium radices atrovirens</i>	—	—	—	2	—	—	—	1	—	—	10	—	—	—	2	—	—	1	—	—	16	
29	<i>Penicillium commune</i>	—	—	—	—	1	—	—	1	—	—	1	—	1	—	2	1	1	—	—	3	11	
30	„ <i>lanoso-coeruleum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	—	—	4	6	
31	„ <i>thomi</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	
32	„ <i>waksmani</i>	—	1	—	1	—	—	—	—	—	—	—	—	1	1	5	1	6	4	8	9	37	
33	<i>Pestalotzia truncata</i>	—	—	—	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
34	<i>Phoma humicola</i>	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	—	—	1	
35	<i>Phoma turviensis</i>	—	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
36	„ sp.	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	
37	<i>Phytophthora similis cactorum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	2	
38	„ sp. 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	33	—	—	—	35	
39	„ „ 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	9	1	—	12	
40	<i>Plenodomus lingam</i>	—	—	—	—	—	—	—	2	—	—	—	5	—	—	—	—	—	—	—	—	7	
41	<i>Pullularia pullulans</i>	—	—	—	1	—	—	—	1	—	—	—	—	—	—	1	—	—	—	—	—	3	
42	<i>Septonema secedens</i>	—	—	—	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	
43	<i>Spicaria griseola</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	
44	„ <i>simplicissima</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	
45	<i>Stemphylium piriforme</i>	1	—	—	1	—	—	1	1	—	—	—	1	—	—	—	—	—	—	2	—	7	
46	<i>Tilachlidium humicola</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	2	
47	<i>Torula allii</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	—	1	
48	„ <i>lucifuga</i>	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	
49	<i>Trichoderma album</i>	—	—	—	6	—	—	—	—	—	—	—	5	—	—	—	—	—	—	—	—	11	
50	„ <i>glaucum</i>	—	—	1	—	2	—	—	1	—	—	—	—	3	—	—	28	5	—	3	5	48	
51	„ <i>koningi</i>	—	—	4	7	—	—	—	11	6	—	9	5	23	—	15	17	33	7	2	19	158	
52	„ <i>lignorum</i>	—	—	—	—	1	—	—	—	—	—	—	—	11	—	—	12	—	—	—	2	26	
53	<i>Verticillium candidulum</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	—	—	2	
54	„ <i>terrestre</i>	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	2	—	—	4	
55	<i>Zygorhynchus vulleumii</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	29	25	54	
56	Grzybnie nie owocujące	5	9	15	6	10	6	21	1	6	10	3	10	6	2	1	4	—	5	—	—	120	
		46	26	44	40	59	29	31	33	65	25	40	44	59	26	33	70	51	79	60	77	937	



Ryc. 6. Turew, stanowisko 2, na glebie torfowej

41. *Plenodomus lingam* v Höhn. — Kolonie były barwy szarobrunatnej, dość puszyste, a na ich powierzchni szczególnie przy szklanych ściankach naczyń tworzyły się liczne, czarne dość duże piknidy. Również wewnątrz pożywki zaobserwowano piknidy. Spód kolonii był ciemnobrunatny. Piknidy wykształcały charakterystyczne, długie, proste lub lekko zgięte ujścia, wysokość piknid wraz z ujściem $864-973 \times 432-453 \mu$, średnica ujścia u nasady $115,4-147,2 \mu$, na szczycie $46-69 \mu$. Strzępki grzybni powietrznej były brunatne, podzielone i gęsto oplatały piknidy. Konidia zaobserwowano owalne lub jajowate, bezbarwne, hialinowe, przeważnie z dwiema kropelkami tłuszczu wewnątrz, $4,6-5,1 \times 1,5-2,3 \mu$.

42. *Pullularia pullulans* (de Bary) Berkhout — Wymiary konidiów $6,9-16,1 (18,4) \times 5,1-7,4 \mu$.

43. *Septonema secedens* Corda — Aksamitne bardzo wypukłe, szarooliwkowe kolonie rosły dość powoli. Konidia tworzyły się w łańcuszkach, brunatne, najczęściej z trzema przegrodami poprzecznymi, $22-25 \times 3,1-5 \mu$.

44. *Spicaria griseola* Sacc. — Wymiary sterygm $5,6-9,2 \times 1,8-2,3 \mu$, konidiów $2,3-2,8 \times 1,5-2 \mu$.

45. *Spicaria simplicissima* Oudemans — Kolonie wyrosły białawe miejscami kremowożółtawe z latami szarozielonymi, zbite, przylegające do podłoża, niskie. Spód kolonii był barwy pożywki. Trzonki

Tabela 7

Wyniki analizy mikologicznej korzeni i gleby wykazujące wspólne gatunki grzybów dla poszczególnych stanowisk

Lp.	Oznaczenie grzyba	Stanowiska			
		1	2	3	4
1	<i>Fusarium solani</i> f. 3	+	+	+	+
2	<i>Fusidium viride</i>	+	+	+	+
3	<i>Hormodendrum resinae</i>	+	+	+	+
4	<i>Penicillium waksmani</i>	+	+	+	+
5	<i>Trichoderma koningi</i>	+	+	+	+
6	<i>Mycelium radidis atrovirens</i>	+	+	+	+
7	<i>Absidia sphaerosporangioides</i>	+		+	+
8	<i>Fusarium martii</i> 1 comb.	+	+		+
9	<i>Mortierella pusilla</i> v. <i>vinacea</i>	+		+	+
10	<i>Penicillium commune</i>	+		+	+
11	<i>Stemphylium piriforme</i>	+		+	+
12	<i>Trichoderma glaucum</i>	+		+	+
13	<i>Cylindrocarpon radicola</i>	+	+		
14	<i>Fusarium conglutinans</i> v. <i>betae</i>	+	+		
15	<i>Fusarium trichothecioides</i>	+	+		
16	<i>Gliocladium deliquescens</i>	+	+		
17	<i>Phytophthora</i> sp. 1	+	+		
18	<i>Verticillium candidulum</i>	+	+		
19	<i>Verticillium terrestre</i>	+	+		
20	<i>Hormodendrum olivaceum</i>			+	+
21	<i>Pullularia pullulans</i>			+	+
22	<i>Zygorhynchus vuillemini</i>			+	+
23	<i>Cephalosporium asperum</i>		+		+
24	<i>Coniosporium arundinis</i>		+		+
25	<i>Fusarium semitectum</i>		+	+	+
26	<i>Fusarium sporotrichioides</i>		+	+	+
27	<i>Penicillium lanoso-coeruleum</i>		+	+	+
28	<i>Phytophthora</i> sp. 2		+	+	+
29	<i>Cladosporium herbarum</i>		+	+	
30	<i>Phytophthora similis cactorum</i>		+	+	
31	<i>Botrytis cinerea</i>			+	
32	<i>Cephalosporium acremonium</i>		+		
33	<i>Chaetomium indicum</i>	+			
34	<i>Coniothyrium bohmani</i>		+		
35	<i>Coniothyrium fuckelii</i>				+
36	<i>Cylindrocarpon didymum</i>		+		

c. d. tab. 7

L. p.	Oznaczenie grzyba	Stanowiska			
		1	2	3	4
37	<i>Hyalopus tener</i>				+
38	<i>Monotospora daleae</i>		+		
39	<i>Mucor hiemalis</i>		+		
40	<i>Mucor ramannianus</i>			+	
41	<i>Penicillium thomi</i>	+			
42	<i>Pestalozzia truncata</i>				+
43	<i>Phoma humicola</i>	+			
44	<i>Phoma turviensis</i>				+
45	<i>Phoma</i> sp.				+
46	<i>Plenodomus lingam</i>				+
47	<i>Septonema secedens</i>				+
48	<i>Spicaria griseola</i>			+	
49	<i>Spicaria simplicissima</i>	+			
50	<i>Tilachlidium humicola</i>			+	
51	<i>Torula allii</i>			+	
52	<i>Torula lucifuga</i>	+			
53	<i>Trichoderma album</i>				+
54	<i>Trichoderma lignorum</i>	+			+
55	<i>Mortierella pusilla</i> v. <i>isabellina</i>				+
		25	27	25	31

konidialne rozgałęziały się w formie pędzelków, wymiary odgałęzień 62,1—82,8 × 1,5—2,3 μ, sterygmy 9,2—13,8 × 2,3—3,3 μ, konidia kuliste, 2,8—3,8 μ.

46. *Stemphylium piriforme* Bonorden — Kolonie wyrastały szaroczarne, puszyste niekiedy całkiem czarne, spód był również czarny. Owocowanie konidialne tworzyło się bardzo obficie, konidia były barwy ciemnobrunatnej, 23—32,2 × 11,5—15 μ.

47. *Tilachlidium humicola* Oudemans — Wymiary główek konidialnych 9,2—16,1 μ, konidioforów 27,6—46 × 2,3—3,3 μ, konidiów 4,6—6,9 × 2,8—3,8 μ.

48. *Torula allii* (Harz.) Sacc. — Kolonie z brzegu jasnoszarobrunatne, w środku przybierały barwę ciemnobrunatną. Strzępki grzybni były bogato rozgałęzione i gęsto podzielone, a na końcach tworzyły długie łańcuszki konidiów, okrągłych, ciemnobrunatnych, z dużą kroplą tłuszczu wewnątrz, 9,2—13,8 μ.

49. *Torula lucifuga* Oudemans — Brunatnoszare kolonie tworzyły na powierzchni pożywki wypukłe poduszczkowate wysepki. Spód kolonii



Ryc. 7. Turew, stanowisko 3, na glebie porolnej

był szaroczarny do czarnego. Strzępki grzybni powietrznej rozgałęziały się słabo, konidia tworzyły się w łańcuszkach, $9,2-20,7 \times 6,9-10,2 \mu$.

50. *Trichoderma album* Preuss — Wymiary konidiów $2,5-3,1 \times 1,8-2,5 \mu$.

51. *Trichoderma glaucum* Abbott — Wymiary konidiów $4,6-5,1 \times 2,3-2,8 \mu$.

52. *Trichoderma koningi* Oudemans — Wymiary konidiów $3,8-4,6 \times 2,3-2,8 \mu$.



Ryc. 8. Turew, stanowisko 4, aleja śródpolna

53. *Trichoderma lignorum* (Tode) Harz. — Wymiary konidiów 2,3—3,3 μ .

54. *Verticillium candidulum* Sacc. — Śnieżno białe, niskie, aksamitne kolonie rosły powoli, tworząc wypukłe wysepki na powierzchni pożywki. Spód kolonii był w środku żółtawy, na brzegach kremowy. Bezbarwne, delikatne trzonki konidialne rozgałęziały się okółkowo, po 3 od-

gałęzienia w okółku, konidia tworzyły się jajowate, nieregularne, hialinowe. Wymiary odgałęzień $16,1\text{--}20,7 \times 1,5 \mu$, konidiów $4,6\text{--}5,1 \times 1,8\text{--}2,5 \mu$.

55. *Verticillium terrestre* (Link) Lindau — Kolonie białe, pajęczynowate, dość gęste, rosły szybko. Trzonki konidialne z czterema odgałęzieniami w okółkach, czasem rozgałęziające się wtórnie również okółkowo. Konidia okrągłe lub eliptyczne, hialinowe, o średnicy $3,1\text{--}5 \mu$ lub $4,3\text{--}5 \times 3,1\text{--}4,3 \mu$.

56. *Zygorhynchus vuillemini* Namysłowski — Kolonie tworzyła szara pajęczynowata grzybnia powietrzna, w której widoczne były, jakby zawieszone drobne, czarne kuleczki. Spód kolonii był barwy pożywki. Zarodnie tworzyły się okrągłe $27,6\text{--}34,5 \mu$, kolumelle okrągłe, $23\text{--}27,6 \mu$, zarodniki zarodniowe $3,8\text{--}4,6 \times 2,3\text{--}2,8 \mu$, zygospori okrągłe, $23\text{--}55 \mu$, chlamydospori okrągłe, $9,2\text{--}11,5 \mu$ i owalne $11,5\text{--}23 \times 9,2\text{--}17,1 \mu$.

Tabela 5 ilustruje rozmieszczenie wyizolowanych grzybów w poszczególnych, przeanalizowanych częściach korzeni oraz glebie z najbliższego ich sąsiedztwa. W pięciu pionowych kolumnach zestawiono cyfry przedstawiające ilości kolonii poszczególnych gatunków grzybów wyizolowanych z pięciu kategorii badanego materiału. Z zestawienia tego wynika, że największej ilości kolonii dostarczyła gleba, w następnej kolejności znalazły się korzenie o średnicy od $1\text{--}3 \text{ mm}$, dalej korzenie do $1 \text{ mm } \phi$, a na końcu mikoryzy i korzenie do $1/2 \text{ mm}$.

Grzybnie nie owocujące, których szczegółowe opisy nie dają właściwie specjalnych korzyści, potraktowano zbiorowo, izolowano je najczęściej z mikoryz i najcieńszych korzeni. Wyraźny spadek ich populacji zaobserwowano w starszych częściach korzeni i w glebie — co wskazywałoby niejako na ich specjalizację. Przypuszczalnie były to grzybnie mikoryzowe, których z braku owocowania nie udało się zidentyfikować.

Tabela 6 orientuje w ilościowym rozdziale wyhodowanych kolonii grzybów, z poszczególnych kategorii analizowanego materiału, na tle czterech stanowisk. Dzięki temu rozbiciu widoczny jest skład gatunkowy grzybów oraz nasilenie ich występowania ilościowego w poszczególnych częściach korzeni i glebie na czterech różnych stanowiskach. Z zestawienia tego wynika, że maksymalną ilość kolonii grzybów otrzymano z mikoryz i korzeni do $1 \text{ mm } \phi$, na stanowisku 1, a minimalną na stanowisku 2. W przypadku korzeni od $1\text{--}3 \text{ mm } \phi$ największej ilości kolonii grzybów dostarczyły materiały ze stanowiska 4, a najmniejszej również ze stanowiska 2.

Ilościowe wyniki izolacji z gleby były większe niż z korzeni, co jest zresztą zrozumiałe, gdyż gleba jest siedliskiem ogromnej ilości mikroorganizmów. Najbogatsza, w obserwowanym przypadku, okazała się gleba

Tabela 8

Rozmieszczenie określonych grzybów na przebadanych częściach korzeni z czterech stanowisk

Lp.	Oznaczenie grzyba	Stanowiska															
		1				2				3				4			
		a	b	c	d	a	b	c	d	a	b	c	d	a	b	c	d
1	<i>Absidia sphaerosporangioides</i>	+	+	+	+							+	+	+		+	
2	<i>Botrytis cinerea</i>									+							
3	<i>Cephalosporium acremonium</i>					+	+										
4	<i>Cephalosporium asperum</i>								+					+		+	
5	<i>Chaetomium indicum</i>		+														
6	<i>Cladosporium herbarum</i>								+	+							
7	<i>Coniosporium arundinis</i>								+								
8	<i>Coniothyrium fuckelii</i>													+			
9	<i>Cylindrocarpon didymum</i>					+											
10	„ <i>radicicola</i>			+		+	+										
11	<i>Fusarium solani</i> f.3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	„ <i>conglutinans</i> v. <i>betae</i>				+			+									
13	„ <i>trichothecioides</i>		+			+	+	+									
14	„ <i>martii</i> f.1		+	+													+
15	„ <i>semitectum</i>					+		+		+					+		
16	„ <i>sporotrichioides</i>							+		+							
17	<i>Fusidium viride</i>	+	+	+		+	+	+	+	+				+	+		
18	<i>Hormodendrum olivaceum</i>									+					+		+
19	„ <i>resinae</i>	+	+	+		+	+		+	+	+			+	+		
20	<i>Mortierella pusilla</i> v. <i>isabellina</i>													+			
21	<i>Mortierella pusilla</i> v. <i>vinacea</i>		+														
22	<i>Mycelium radicis atrovirens</i>											+	+	+	+		
23	<i>Penicillium commune</i>		+		+							+	+		+		+
24	„ <i>lanoso-coeruleum</i>								+			+					
25	„ <i>waksmani</i>				+	+			+			+	+				+
26	<i>Pestalozzia truncata</i>														+		
27	<i>Phoma humicola</i>				+												
28	<i>Phoma turviensis</i>													+			
29	<i>Plenodomus lingam</i>														+	+	
30	<i>Pullularia pullulans</i>											+	+	+	+		
31	<i>Septonema secedens</i>											+					
32	<i>Spicaria griseola</i>											+					
33	<i>Stemphylium piriforme</i>	+										+	+	+	+	+	
34	<i>Torula allii</i>											+					
35	„ <i>lucifuga</i>	+															
36	<i>Trichoderma album</i>													+		+	
37	„ <i>glaucum</i>		+		+					+					+		+
38	„ <i>koningi</i>			+	+					+		+	+	+	+	+	+
39	„ <i>lignorum</i>		+		+												+
40	<i>Verticillium terrestre</i>	+															
		7	11	7	9	6	7	4	11	8	5	6	10	15	13	7	8

a — mikoryzy, b — korzenie do 1/2 mm Ø, c — korzenie do 1 mm Ø, d — korzenie do 3 mm Ø.

Tabela 9

Grzyby wyizolowane wyłącznie z gleby z rozbiem na cztery stanowiska

Lp.	Oznaczenie grzyba	Stanowiska			
		1	2	3	4
1	<i>Coniothyrium bohmani</i>		+		
2	<i>Gliocladium deliquescens</i>	+	+		
3	<i>Hyalopus tener</i>				+
4	<i>Monotospora daleae</i>		+		
5	<i>Mucor hiemalis</i>		+		
6	<i>Mucor ramannianus</i>			+	
7	<i>Penicillium thomii</i>	+			
8	<i>Phoma</i> sp.				+
9	<i>Phytophthora similis cactorum</i>		+	+	
10	<i>Phytophthora</i> sp. 1	+	+		
11	<i>Phytophthora</i> sp. 2		+	+	+
12	<i>Spicaria simplicissima</i>	+			
13	<i>Tilachlidium humicola</i>			+	
14	<i>Verticillium candidulum</i>	+	+		
15	<i>Zygorhynchus vuillemini</i>			+	+
		5	8	5	4

torfowa, najuboższa na śródleśnej polanie. Z gleby wyizolowano mało grzybní nie owocujących i tylko na stanowisku 2.

Tabela 7 przedstawia wspólne gatunki grzybów dla poszczególnych stanowisk. Najwięcej analogicznych gatunków znaleziono na stanowiskach 3 i 4 oraz 1 i 2. Stanowiska 3 i 4 znajdowały się na glebach, które możemy określić jako rolnicze, gdyż stanowisko 3 (ryc. 7) było przed wysadzeniem topoli w użytkowaniu rolniczym, a stanowisko 4 (ryc. 8) jest aktualnie skrajem pól uprawnych. W przypadku stanowisk 1 (ryc. 5) i 2 (ryc. 6) gleby można określić jako leśne ze względu na użytkowanie i otoczenie.

Ponadto dużą ilość wspólnych gatunków znaleziono na stanowiskach 2 i 3. Stanowisko 2 jest zagajnikiem w otoczeniu leśnym podobnie jak 3 — z tą jednak różnicą, że ostatnie było w użytkowaniu rolniczym przed wysadzeniem topoli.

Tabela 8 orientuje w rozmieszczeniu określonych grzybów w poszczególnych, przeanalizowanych częściach systemu korzeniowego topoli na tle czterech stanowisk. Z tabeli tej wynika, że najwięcej różnych

gatunków wyizolowano z mikoryz i korzeni do 1/2 mm ϕ na stanowiskach 1 i 4. Nieco mniejszą, ale jednakową ilość gatunków z tych samych części korzeni na stanowiskach 2 i 3. Dość dużą i jednakową ilość gatunków wyizolowano na stanowiskach 1 i 3 z korzeni starszych od 1 do 3 mm ϕ , również jednakową, lecz nieco mniejszą od poprzedniej otrzymano ze stanowisk 2 i 4.

Najbogatszy skład gatunkowy cechował korzenie topól najlepiej rozwiniętych na stanowisku 4, przy alei. Najuboższy na stanowisku 2, na glebie torfowej, gdzie drzewa rosły źle, a nawet zamierały. Gdyby dodano do przedstawionych cyfr ilość kolonii grzybnii nie owocujących wzrosłyby jedynie liczby, ale stosunek ich nie uległby zasadniczej zmianie.

Tabela 9 przedstawia grzyby wyizolowane wyłącznie z gleby z roz biciem na cztery stanowiska.

DYSKUSJA WYNIKÓW

W przedstawionej pracy, przy zastosowaniu tej samej co w odniesieniu do świerka metody badawczej (Mańka i Truszkowska 1958) i wykonaniu dodatkowo izolacji z gleby w najbliższym sąsiedztwie korzeni, zapoznano się chociażby częściowo z grzybami współżyjącymi z systemem korzeniowym topól, rosnących w określonych siedliskach.

Niewiele przybyło wiadomości na temat grzybów mikoryzowych topoli. Jedynie nie owocujące grzybnie ze sprzążkami, a więc *Basidiomycetes*, wyizolowane z mikoryz, pozwalają przypuszczać, że były one i w tym przypadku symbiontami. Pozostałe określone do gatunku grzyby, wyizolowane również z mikoryz, mogą być grzybami mikoryzowymi lub im towarzyszącymi, czego nie da się wyjaśnić na podstawie przeprowadzonych dociekań.

W wyniku izolacji z gleby okazało się, że szereg grzybów występujących w najbliższym sąsiedztwie korzeni, w danym przypadku nie nawiązał z nimi bezpośrednich kontaktów. Opracowany skład populacji grzybów glebowych był podobny do wyników podanych przez Waksmana (Warcup 1959) wykazujących, że w glebie dominują rodzaje: *Mucor*, *Penicillium*, *Trichoderma* i *Aspergillus*, gdyż najczęściej otrzymywano je w wyniku izolacji. Towarzyszą im często: *Rhizopus*, *Zygorhynchus*, *Fusarium*, *Cephalosporium* i *Verticillium* oraz wg najnowszych badań *Absidia*, *Gliocladium* i *Chaetomium*. Rodzaj *Fusarium* zdaniem Warcupa (1959) należy do grzybów bardzo często izolowanych z gleby, ale w większości przypadków są to gatunki saprofityczne, a nie patogeny roślin, co potwierdzają przedstawione badania.

Grzyby, wchodzące w kontakty anatomiczne z okrywającymi tkankami starszych korzeni, można by podzielić na dwie grupy: pasożyty

fakultatywne i saprofity zasiedlające zamierającą tkankę przeznaczoną do złuszczenia.

Przykładem grzyba, którego można potraktować jako pasożyta, na podstawie przeprowadzonej analizy był *Pestalotzia truncata*, wyizolowany z najdelikatniejszych, najmłodszych części badanych korzeni do 1/2 mm ϕ , bezpośrednio nad mikoryzami. Wybór tego stosunkowo najłatwiejszego, ze względu na delikatność młodej tkanki, miejsca jako punktu wejściowego może wskazywać na pasożytnicze właściwości. Podobnie można uzasadnić występowanie *Coniothyrium fuckelii*, który zazwyczaj spotykano na organach nadziemnych roślin (Grove 1937) jako pasożyta ran lub saprofita, ale który znany był również z występowania w glebie (Gilman 1959).

W danym przypadku, podobnie jak w odniesieniu do świerka, niewątpliwie całość wyizolowanych grzybów tak z systemu korzeniowego jak z gleby jest „grupą o określonym ekologicznym uwarunkowaniu i o określonej biologicznej funkcji“ (Mańka i Truszkowska 1958), która może mieć wpływ na zdrowotność korzeni topól, a co za tym idzie na ich rozwój i przyrosty.

Pośród badaczy grzybów glebowych część interesowała się przede wszystkim stanem ilościowym populacji mikroorganizmów glebowych, byli jednak wśród nich i tacy, którzy obserwowali skład gatunkowy tych populacji, a jeszcze inni śledzili procesy chemiczne zachodzące w glebie na skutek obecności różnych mikroorganizmów oraz ich wpływu na żyzność gleby i wzrost roślin (Waksman 1937). Przedstawiona praca była nastawiona przede wszystkim na poznanie składu gatunkowego grzybów współżyjących z topolą w określonych warunkach siedliskowych, co mogłoby wykazać interesujące związki między rozwojem drzew a formą zagrzybienia ich korzeni.

Fragmentaryczne badania flory grzybów, związanych ze zdrowymi korzeniami tychże topól, przeprowadzone w 1955 r. wspólnie z K. Mańką, nie zostały w aktualnej pracy wykorzystane, gdyż w międzyczasie zamarły nie oznaczone kolonie grzybów. Niemniej, już wtedy, na podstawie nie określonych jeszcze do gatunku grzybów, różnicowały się poszczególne siedliska.

WNIOSKI

1. Z przeprowadzonej analizy mikologicznej najmłodszych części systemu korzeniowego topoli (*Populus euramericana marilandica* Bosc.) oraz gleby z ich sąsiedztwa wynika, że stosując przedstawione w tekście metody badawcze, największą ilość grzybów otrzymano z mikoryz i korzeni do 1/2 mm ϕ w przypadku drzew ze stanowiska 4 (28 gatunków),

a trochę mniejszą ze stanowiska 1 (18 gatunków). Najmniejszą i jednokową ilość gatunków uzyskano ze stanowisk 2 i 3 (13 gatunków).

2. Z korzeni starszych, o grubości od 1—3 mm ϕ , najwięcej grzybów wyizolowano w przypadku stanowisk 1 i 3 (po 16 gatunków), a o jeden mniej z 2 i 4.

3. Poszczególne części przebadanych korzeni różniły się nieco między sobą składem gatunkowym mikoflory nawet w obrębie jednego stanowiska. Ponadto mikoflora korzeniowa zróżnicowała cztery stanowiska topoli w Turwi.

4. Większość grzybów występowała jednocześnie w różnych częściach badanych korzeni. Na specjalną uwagę zasługuje gatunek *Fusarium solani* f. 3, który wyizolowano ze wszystkich części badanych korzeni.

5. *Mycelium radialis atrovirens* Melin, szczególnie licznie zasiedlający korzenie świerka (512 kolonii), w przypadku topoli okazał się rzadko spotykanym gatunkiem, gdyż wyizolowano tylko 16 kolonii.

6. Na 33 określone gatunki grzybów z gleby — 17 nie nawiązywało, w danych warunkach, bezpośrednich kontaktów z korzeniami.

7. Pobieżna analiza mikologiczna gleby w zasięgu korzeni topoli wskazała również na różnice w składzie gatunkowym mikoflory poszczególnych stanowisk w Turwi.

8. Najwięcej wspólnych gatunków grzybów zaobserwowano na stanowiskach 3 i 4, czyli na glebach rolniczych oraz 1 i 2, czyli na glebach leśnych.

9. Siedlisko wykazujące dużą różnorodność mikoflory oraz warunki umożliwiające nawiązywanie bezpośrednich kontaktów przez korzenie drzew wydaje się korzystne dla topoli, czego dowodem są najlepiej rozwinięte drzewa alejowe.

10. W przypadku gleby torfowej, pomimo bagactwa mikoflory, stwierdzono słabe zagrzybienie korzeni topoli i zły rozwój drzew, a nawet ich zamieranie.

11. Wyizolowane z materiału korzeniowego i gleby grzyby potraktowano ogólnie jako zespół grzybów korzeniowych topoli uzależniony w swej jakościowej i ilościowej formie od zastosowanej metody badawczej.

12. Oznaczono dwa dotąd nie znane gatunki grzybów: *Coniothyrium bohdani* i *Phoma turviensis*.

Poczuwam się do miłego obowiązku podziękowania na tym miejscu P. P. Kołegom: mgr J. Narkiewiczowi-Jodko i inż. A. Pacanowskiemu z Turwi za pomoc przy zbieraniu materiału oraz mgr St. Wnekowskiemu za wykonanie zdjęć fotograficznych stanowisk topoli.

(Wpłynęło dn. 25.1.1961)

Z Katedry Filopatologii WSR we Wrocławiu
i Pracowni Mikologicznej PAN w Warszawie

RÉSUMÉ

Le présent travail concerne les observations effectuées sur la microflore fongique des radicelles et de jeunes racines du peuplier (*Populus euramericana marilandica* Bosc.) de 4 diverses plantations à Turew près de Poznań en Pologne. Les plantations mentionnées ont été situées dans 4 divers lieux; la 1^e — était située sur l'éclaircie forestière entourée de bois de pins, la 2^e — sur la tourbière entourée de bois d'aunes, la 3^e — était située sur le sol cultivé autrefois, la 4^e — formait une allée au milieu des champs cultivés. Les arbres observés se laissaient caractériser par de grandes différences de hauteur et de diamètre du tronc. A cause de ces différences on a d'abord examiné les quatres milieux où poussaient les peupliers et ensuite la santé des arbres et la dynamique du développement de leurs mycorrhizes (Wilusz, 1953, (Mme) Truszkowska et Narkiewicz-Jodko, 1955). Actuellement on a fait l'analyse mycologique des radicelles transformées en majeur partie en mycorrhizes, jeunes racines jusqu'à 3 mm de diamètre et également l'analyse du sol dans leur voisinage à l'aide de la méthode des cultures, artificielles, pures.

On a déterminé 53 espèces et 3 genres de champignons et on a cultivé 36 cultures de différents myceliums stériles, c'est-à-dire ne fructifiant que sur les gélés nutritifs appliqués.

La plus grande diversité des champignons a été isolée de mycorrhizes et de radicelles jusqu'à 1/2 mm de diamètre. Cette diversité diminuait dans les racines plus âgées. On a isolé du sol beaucoup de champignons de la même espèce que des racines.

On a trouvé que la majorité des cultures de champignons provenait de mycorrhizes des arbres dans la plantation sur l'éclaircie forestière entourée de bois de pins, la minorité-provenait des arbres dans la plantation sur la tourbière entourée de bois d'aunes. La même situation a été constatée dans les radicelles de 1/2 à 1 mm de diamètre. Par contre les racines plus âgées de 1 à 3 mm de diamètre avaient pourvu à la majorité de cultures des champignons dans la plantation en forme d'une allée champêtre — la minorité des champignons on a constatée sur la tourbière. Chaque plantation du peuplier parmi celles qu'on a observé à Turew était caractérisée par une composition spécifique et différente de la flore fongique des racines et du sol mais il y avait toujours un certain nombre d'espèces communes pour toutes les quatres plantations. Il y avait spécialement beaucoup d'espèces communes dans les deux milieux forestiers, ainsi que dans les deux milieux agricoles.

Le développement du peuplier dans chaque plantation à Turew dépendait probablement, entre autres, de la composition de la flore fongique.

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Genetic Studies on the genus *Aquilegia* L.

II. Crosses between *Aquilegia ecalcarata* Maxim. and *Aquilegia chrysantha* Gray

W. PRAŽMO

This second paper on the experiments with the genus *Aquilegia* reports the results of genetic analyses of the crosses between *A. ecalcarata* and *A. chrysantha*. There are many reasons to assume that *A. ecalcarata* is a relic species closely resembling the primitive plants from which the whole genus *Aquilegia* originated (W. Pražmo 1960). *A. chrysantha* belongs to the *caerulea* complex (Grant 1952), which probably differentiated in North America and is pollinated by moths.

A. chrysantha has very long spurs and in this regard diametrically differs from *A. ecalcarata*, which has no spurs. Since the main trend in the differentiation of *Aquilegia* species has been towards morphological differences in the flower structure (Clausen, Keck, and Hiesey 1945) which reflect the adaptation of the particular species to the different modes of pollination (Grant 1952), this investigation has been primarily concerned with the analysis of the morphological traits of flowers.

MATERIAL AND METHODS

The plants of *A. ecalcarata* (P_e) are 15 to 30 cm. high; they have triangular leaves and small purple flowers drooping on very slender peduncles (Fig. 1). The sepals of the calyx are, elliptically ovoid, and pointed at the tip. The petals of the corolla are narrow and elongated, rounded at the tip, spurless, and bent at the base into a bag-like process. The species comes from China.

The plants of *A. chrysantha* (P_{ch}) are taller, 60 to 90 cm. high, with bright green triangular leaves and large, erect, pale yellow flowers on stiff peduncles (Fig. 2). The sepals of the calyx are ovate-lanceolate. The petals of the corolla are oblong-ovate, bluntly rounded at the tip, and parted. The spurs are very long (37 to 46 mm.), parted fairly wide at the base, gradually passing to a narrow tube slightly thicker at the end (the nectary). The stamens stand well above the petals of the corolla.



Fig. 1. *Aquilegia ecalcarata*



Fig. 2. *Aquilegia chrysantha*

A. chrysantha comes from the South-East of the United States of North America.

The parent plants for the crosses were obtained by self-pollination of individuals of both species.

The pollination of *A. ecalcarata* by pollen of *A. chrysantha* produced well developed follicles containing seeds that germinated very well. In spite of several attempts all reciprocal crosses were unsuccessful. The first hybrid generation of 22 plants was uniform with regard to the traits considered in the investigation. All the hybrid individuals began flowering simultaneously with *A. ecalcarata* (between May 17 and 20). *A. chrysantha* began flowering a fortnight later (May 29 to June 2).

After seven or eight days when the hybrids were in full blossom they were self-pollinated or inter-pollinated so as to obtain seeds for the F_2 generation. The setting of seeds in isolating bags without additional pollination was 4.3 per cent and when additional pollination was repeated several times the setting of seeds increased to 8 per cent. The total number of collected seeds was 2097. The seeds were sown out into boxes in the spring of the following year. After four to five weeks 630, i.e. 30 per cent, of the seeds germinated. At the stage of two leaves the seedlings were transferred into pots and three months later they were planted out into experimental plots. A certain number of seedlings (21 per cent) perished soon after germination.

For pollen fertility counts pollen grains were stained with 1 per cent aceto-carmin. Buds collected for examinations of meiosis in pollen mother cells were fixed in Carnoy's fixative. The preparations were made with the smear technique and stained with 1 per cent aceto-carmin or 1 per cent propio-carmin. The colours of flowers were determined according to the Horticultural Colour Charts I and II.

RESULTS

Cytological Analysis

A. ecalcarata, *A. chrysantha*, and the first generation of crosses all have the haploid chromosome number $n=7$. From each of the three groups of plants 20 PMCs were examined in the stages of metaphase, meta-anaphase, and late anaphase of the first division. In the metaphase of the first meiotic division there were always seven bivalents. The segregation of chromosomes in anaphase was always undisturbed (Fig. 3).

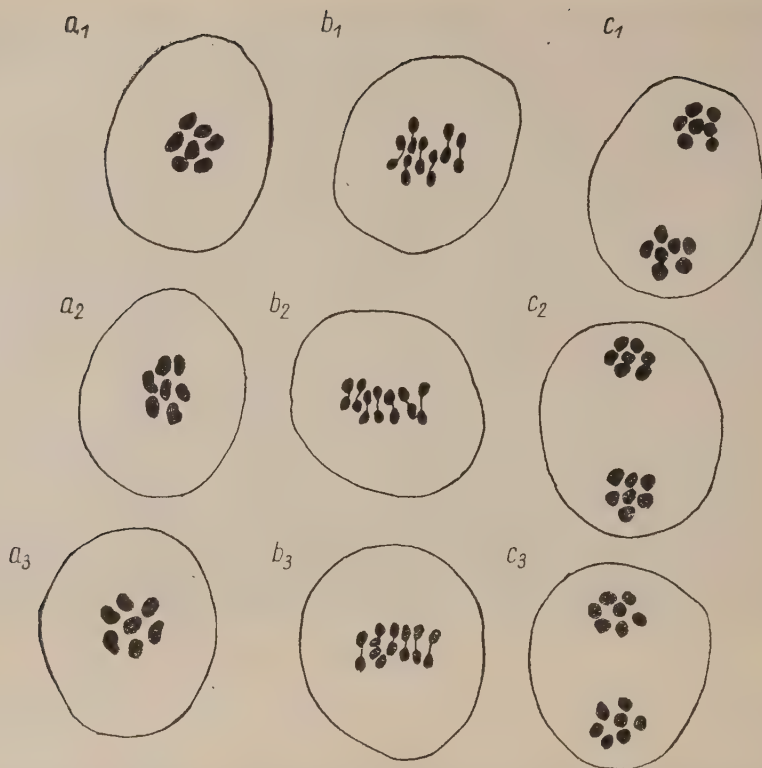


Fig. 3. Meiosis in PMCs of *A. ecalcarata* (a_1, b_1, c_1), *A. chrysantha* (a_2, b_2, c_2), and the first hybrid generation of the cross *A. ecalcarata* $\times$ *A. chrysantha* (a_3, b_3, c_3): a_1, a_2, a_3 — first metaphase, b_1, b_2, b_3 — first meta-anaphase, c_1, c_2, c_3 — first telophase

The inheritance of traits distinguishing the two parental species

The following traits were considered in the genetic analysis of the crosses of *A. ecalcarata* with *A. chrysantha*:

- 1) height of plants,
- 2) shape of leaves,
- 3) length of spurs,
- 4) length of petals of the corolla,
- 5) width of petals of the corolla,
- 6) length of sepals of the calyx,
- 7) width of sepals of the calyx,
- 8) colour of flowers,
- 9) growth habit of flowers,
- 10) length of follicles,
- 11) number of ovules in follicle,

- 12) seed fertility,
- 13) size of seeds,
- 14) pollen fertility.

The correlation between some of these traits was also studied.

1. Height of plants. The average height of *A. ecalcarata* was 19.7 cm. $\pm$ 0.51 and of *A. chrysantha* 74.0 cm. $\pm$ 2.04. As is to be seen the difference was considerable. The height of the first hybrid generation was intermediate: $\bar{x}$ = 55.7 cm. $\pm$ 1.19. In the second hybrid generation

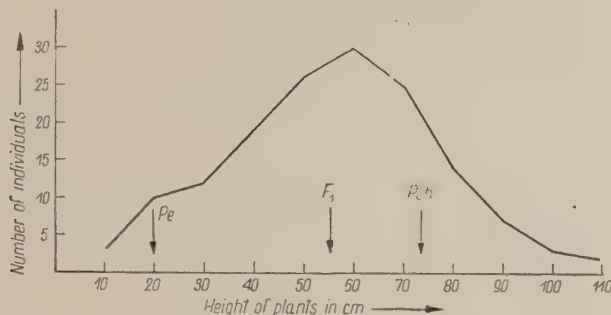


Fig. 4. Curve showing the variation of the height of plants in F_2 ($N = 152$) of the hybrid *A. ecalcarata* $\times$ *A. chrysantha*

of 152 individuals there were plants approaching the height of either of the parental species, but plants of approximately the same height as in F_1 predominated (Fig. 4).

2. Shape of leaves. The leaves in *A. ecalcarata* and *A. chrysantha* were very similar. In both parental species the leaves were thrice odd-pinnate and the leaflets of the 1st and 2nd order were tripartite and bilobate. As is to be seen in fig. 5 the leaves of *A. ecalcarata* were more deeply incised. The first hybrid generation had leaves almost exactly identical with *A. chrysantha* (Fig. 5). Owing to this in F_2 only two classes



Fig. 5. Basal leaves of *A. ecalcarata* — P_e , of *A. chrysantha* — P_{ch} , and of F_1 plants

were distinguishable and even so with difficulty. In one class were the plants with leaves resembling *A. ecalcarata* and in the other the plants with leaves like in *A. chrysantha* and F_1 . Of the 310 F_2 plants 62 were of the first class and 248 of the second.

Table 1
Length of spurs in mm.

Medium class value	0	2	5	8	11	14	17	20	23	26	29	32	35	38	41	44	$\bar{x}$	$\pm s_{\bar{x}}$	N
P_e	20																		20
P_{ch}														5	7	8	41.4	0.56	20
F_1								5	11	4							22.8	0.35	20
F_2	17	35	24	16	20	36	31	34	41	13	12	5					15.2*	0.51	284

* Mean $\bar{x}$ for F_2 with $N=284-17=267$

3. Length of spurs. The most important trait distinguishing the two species was the presence or the absence of the spur and also the length of the spur. Table 1 lists the length of spurs in *A. chrysantha* and in F_1 as well as the variation range of this trait in F_2 . With regard to the length of spurs the first hybrid generation was intermediate. Among the 284 individuals in F_2 there were 17 with spurless flowers and 267 plants had flowers with spurs of various length. Fig. 6 shows the petals (the spur with the petal) arranged in the decreasing order of spur lengths from 33 mm. to 0 mm. The ratio 17 to 267 corresponds to the Mendelian ratio of 1 to 15 which shows that there can be two dominant genes responsible for the presence of spurs in *Aquilegia chrysantha*. The distribution of the variation of the spur length in F_2 (Table 1) markedly departs from the usual relations. The number of individuals in the particular classes is very much the same and the drop of the curve begins only at the point where the length of spurs is about 26 mm. This curve cannot be interpreted as having a normal shape.

4. Length of petals of the corolla. In both parental species the length of petals was very similar. In the first hybrid generation the variation range and the mean values for this trait were almost the same as in *A. chrysantha* (Table 2). The variation range was greater in F_2 than in the parental species and in F_1 , but owing to the excessive differences between the sizes of the particular populations the evidence was insufficient to suppose that transgression had occurred. The variation in F_2 was continuous, which could indicate the presence of polymeric genes.

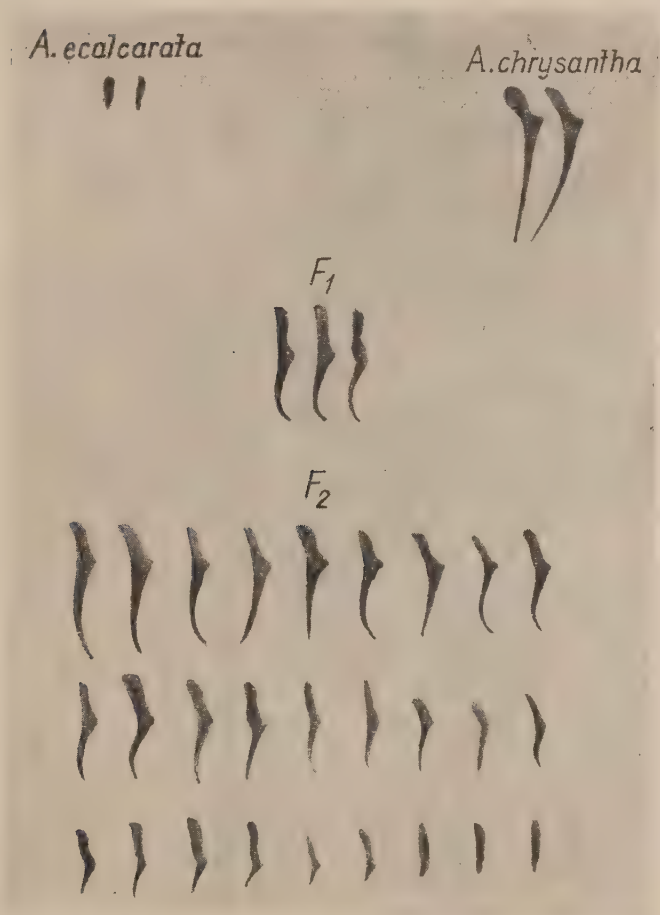


Fig. 6. Photographs of the petals of the corolla in the parental species, in F_1 and in F_2

5. Width of petals of the corolla. The petals of *A. chrysantha* were much wider than of *A. ecalcarata*. In this regard the first hybrid generation was distinctly intermediate and the variation in the second hybrid generation was continuous (Table 3).

6 and 7. Length and width of sepals of the calyx. The variation of these two traits in the investigated plants is shown in tables 4 and 5. As is to be seen so far as these traits are concerned the first hybrid generation clearly resembled *A. chrysantha*. The same data indicate that the shape of the sepals in both parental species differed distinctly. The ratio of the length of sepals to their width was accepted as the index reflecting the shape of sepals. The data in Table 6 show the variation range of the shape of sepals in *A. ecalcarata*, *A. chrysantha*, F_1 ,

Table 2

Length of corollas blade in mm.

Class value	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	$\bar{x}$	$\pm S_{\bar{x}}$	N
P _e						1		2	7	7	3							14,4	0,23	20
P _{ch}								1	2	7	5	2		2	1			15,75	0,34	20
F ₁								2	2	8	7		1					15,2	0,25	20
F ₂	2		4	10	10	22	25	45	39	42	28	26	17	7	3	3	1	13,1	0,34	284

Table 3

Breadth of corollas blade in mm.

Class value	3,5	4,5	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	$\bar{x}$	$\pm S_{\bar{x}}$	N
P _e				3	8	8	1					7,0	0,19	20
P _{ch}							5	14	1			9,8	0,12	20
F ₁					2	3	11	3	1			8,9	0,21	20
F ₂	5	19	43	54	73	56	23	9	1	1		7,7	0,09	284

Table 4
Length of sepals in mm.

Class value	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	22,5	23,5	24,5	25,5	26,5	$\bar{x}$	$\pm s_{\bar{x}}$	N
P _e									3	8	5	2	2	4		4	4	1				15,6	0,39	20
P _{ch}										2	2	2	3	4		4						19,5	0,50	20
F ₁										1	3	4	3	2	2	6	1					19,2	0,39	20
F ₂	3	2		6	9	10	14	10	15	22	20	23	24	40	29	26	13	11	4	1	2	18,4	0,71	284

Table 5

Breadth of sepals in mm.

Class value	2,5	3,5	4,5	5,5	6,5	7,5	8,5	9,5	10,5	11,5	12,5	13,5	14,5	15,5	16,5	$\bar{x}$	$\pm s_{\bar{x}}$	N
P _e		1	6	9	4											5.8	0.16	20
P _{ch}				1	3	5	1	5	4						1	9.0	0.46	20
F ₁					5	6	4	4								8.0	0.27	20
F ₂	3	22	34	45	46	53	46	22	7	5	1					7.3	0.11	284

Table 6

The values of the coefficient of shape of the sepals (1/b)

Class value	14,5	15,5	16,5	17,5	18,5	19,5	20,5	21,5	22,5	23,5	24,5	25,5	26,5	27,5	28,5	29,5	30,5	31,5	32,5	33,5	34,5	$\bar{x} \pm$	$s_{\bar{x}}$	N
P _e								2		1		3	1	4	4	2	1				2	2.71	0.08	20
P _{ch}	1	1	1	1	3	4	1			1	1		1	2	1	1						2.20	0.35	20
F ₁			1	2	2	1				3	1	1	3	1			3					2.32	0.30	20
F ₂	2		3	5	8	12	23	30	33	29	35	34	25	15	10	4	8	2	2	4		2.45	0.67	284

and F_2 . The shape of sepals in the hybrid generation was intermediate and in F_2 the range of variation did not extend beyond the parental types.

8. Colour of flowers. *A. ecalcarata* had purple flowers. According to the Horticultural Colour Chart the corolla of this species was lilac purple 031/2 and of the calyx pansy violet 055. The colour of these flowers was caused by anthocyanin present in the cell sap. The flowers of *A. chrysantha* were yellow. The colour of both petals and sepals was aureolin 3/1 and of the spurs barrium yellow 503/2. The colour of the flowers was due to the presence of chromoplasts. The first hybrid generation was uniform and had flowers of two colours. The sepals of the calyx and the spurs were lilac purple 131/4; the petals of the corolla were mimosa yellow 602/1 over 2/3 of their surface on the inside and the outside along the margins and lilac purple 031/2 on the outside. With regard to the colour of flowers the 275 individuals in F_2 were divided into four classes.

The first class was composed of plants with flowers coloured similarly as in F_1 . The colour of the sepals and spurs in these plants was always of various shades of purple or violet. The petals had different colour patterns: 1) 25 individuals had petals all yellow; 2) 33 individuals had petals purple (various shades of purple) on the outside and all yellow or yellow over 1/3 or 2/3 of the surface on the inside; 3) 69 individuals had petals partly yellow (e.g. over 1/3 of the surface) both on the outside and the inside. Altogether there were 127 plants with flowers coloured similarly as in F_1 .

The second class was composed of plants with yellow flowers resembling *A. chrysantha*. The colour in various individuals was of different intensity from pale yellow to dark yellow. The sepals and the spurs always were somewhat darker than the petals. The number of these plants was 46. This class also comprised 12 plants with pale creamy or cream-white flowers.

The third class was composed of plants with flowers all pink or having pink sepals and yellow petals. The intensity and the shade of these colours varied. The class consisted of 56 plants.

The fourth class comprised individuals in which the colour of the flowers was determined by the presence of anthocyanin only. The sepals and the petals of the flowers in these plants were of various shades and intensities of purple or violet. There were 34 plants in this group. These results are analysed in the discussion further on.

9. Growth habit of flowers. *A. ecalcarata* had drooping flowers, whereas in *A. chrysantha* they were distinctly erect (fig. 1 and 2). In the first hybrid generation all the plants had drooping flowers, and in the second hybrid generation consisting of 275 individuals there were

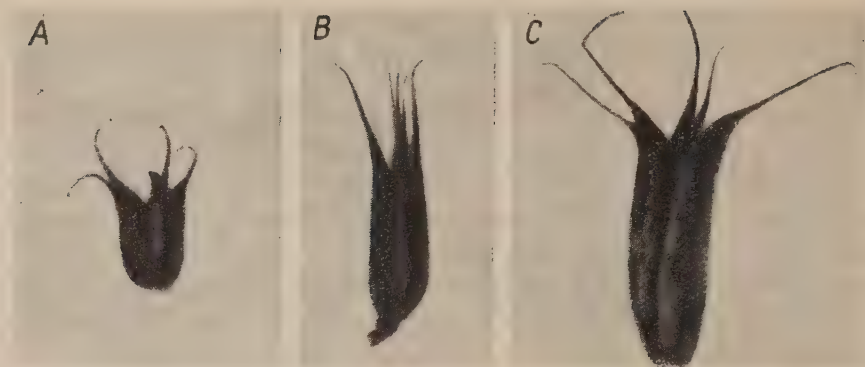


Fig. 7. Photographs of the follicles in the parental species and in F_1 . A — *A. ecalcarata*; B — F_1 generation of *A. ecalcarata* $\times$ *A. chrysantha*; C — *A. chrysantha*

209 plants with drooping flowers and 66 with erect flowers. This means that the segregation was monogenic according to the ratio 3:1 (the anticipated numbers were 206.25 : 68.75).

10. Length of follicles. The two parental species differed greatly by the length of their follicles (Fig. 7). In *A. ecalcarata* the length of follicles was 9.0 mm. $\pm$ 0.29 and in *A. chrysantha* 25.4 mm. $\pm$ 0.87. With regard to this trait the first hybrid generation resembled *A. ecalcarata* the mean length of follicles in F_1 plants being 12.8 mm. $\pm$ 0.32. Figure 8 shows the frequency distribution in the second hybrid generation of 150 individuals. The curve has a very regular shape with the peak at 12 mm. and slightly shifted towards *A. ecalcarata*. In view of the continuous nature of the variation the presence of polymerous genes can also in this case be assumed.

11. Number of ovules in follicles. The counts of the mean number of ovules in follicles included the number of unfertilized ovules, of ovules fertilized but undeveloped, and of normally developed seeds. In *A. ecalcarata* the number of ovules per follicle was 19.2, in *A. chrysantha* 40.3, and in the first hybrid generation 24.95. Figure 9 shows the variation of this trait in the F_2 generation composed of 150 individuals. The peak of the curve is shifted towards *A. ecalcarata*. The curve has both arm sloping regularly and almost symmetrically. The continuous nature of the variation seems to be indicative of the polygenic mechanism in the heredity of ovule numbers.

12. Seed fertility. The counts for seed fertility were made on follicles obtained from free-pollinated flowers. For calculating seed fertility the ratio of well developed seeds to the total number of ovules in a follicle was determined. The proportion of well developed seeds in

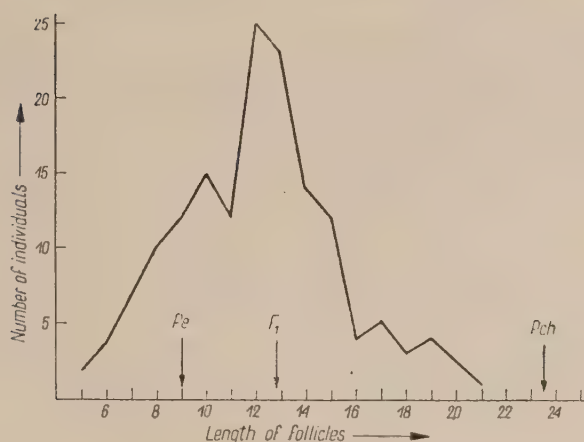


Fig. 8. Frequency distribution curves showing the length of follicles in 150 plants of the F_2 generation of *A. ecalcarata* $\times$ *A. chrysantha*

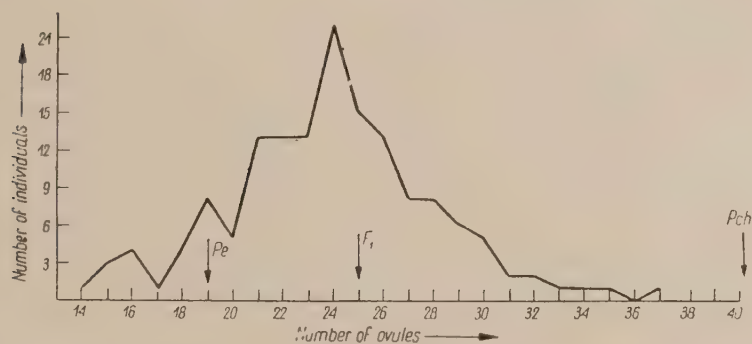


Fig. 9. Curve showing the variation in the number of ovules in the F_2 generation of the hybrid *A. ecalcarata* $\times$ *A. chrysantha*

A. ecalcarata ranged 52 to 60 per cent, average 57 per cent, and in *A. chrysantha* 30 to 42 per cent, average 36 per cent. Undeveloped ovules were found in neither of the parental species, but in the hybrids the inhibited development of ovules seemed rather common. In F_1 fertility

Table 7

Seed fertility in % in F_2

Class value	0	1	10	20	30	40	50	60	70	80	90	100	N
Number of plants	7	20	44	32	25	6	13	3					150

Table 8
Length of the seeds in mm

Class value	1,05	1,15	1,25	1,35	1,45	1,55	1,65	1,75	1,85	1,95	2,05	2,15	2,25	2,35	2,45	2,55	2,65	2,75	2,85	2,95	3,05	3,15	3,25	$\bar{x}$	$\pm s_{\bar{x}}$	N	
P _e	4	7		5	4												4	5	1				1		1,30	0,035	20
P _{ch}																	4								2,79	0,04	20
F ₁											1		9	7		3									2,34	0,027	20
F ₂				1	2	1	5	9	9	22	23	21	24	15	4	3	2	2							2,20	0,34	145

Table 9
Breadth of the seeds in mm

Class value	0,55	0,65	0,75	0,85	0,95	1,05	1,15	1,25	1,35	1,45	1,55	1,65	$\bar{x}$	$\pm s_{\bar{x}}$	N
P _e	4			9	4	3							0,79	0,032	20
P _{ch}										9	10	1	1,45	0,020	20
F ₁							13		7				1,26	0,021	20
F ₂	1	1		2	9	8	32	39	28	16	8	1	1,27	0,560	145

dropped to 8.3 per cent; the variation of seed fertility in the F_2 generation is shown in table 7. Individuals with seed fertility ranging 15 to 20 per cent were the most numerous. Some individuals in F_2 were completely sterile, whereas others were more fertile than the parental plants.

Table 10

Pollen fertility in % in F_2

Class value	0	10	20	30	40	50	60	70	80	90	100	N
Number of plants	12	12	23	25	31	45	28	22	20	4		222

13. Size of seeds. Simultaneously to the counts of the well developed seeds the length and width of seeds was measured, since it was noticed that the size of seeds in the two parental species considerably differed. Tables 8 and 9 list the values of the variation range and the mean values for the length and width of seeds in *A. ecalcarata*, *A. chrysantha*, F_1 , and F_2 . In both instances the mean values in F_1 approached the values for *A. chrysantha*. In F_2 variations were continuous.

14. Pollen fertility. In the parental species *A. ecalcarata* and *A. chrysantha* pollen fertility was 87 and 83 per cent respectively. In the first hybrid generation pollen fertility ranged 54 to 77 per cent, average 62 per cent. As is to be seen the drop of pollen fertility in F_1 was small. The variation of this trait in F_2 is shown in table 10. Among the 222 individuals of the F_2 generation 24 plants had pollen fertility above 80 per cent and many plants had a greatly reduced pollen fertility.

CORRELATIONS

The photograph of the petals of the corolla (fig 6) in *A. ecalcarata*, *A. chrysantha*, and the F_1 and F_2 crosses between these two species seemed to indicate that there was no direct correlation between the length of petals and the length of spurs. To check this supposition the correlation between these two traits was examined. The coefficient of correlation between the length of spurs and the length of petals was $r = -0.1174$ in *A. chrysantha*, $r = -0.0315$ in F_1 , and $r = -0.0789$ in F_2 . These values indicate that there was no correlation whatever between the two traits (the size of the populations in the first two instances was $n = 100$ and in F_2 $n = 267$).

On the other hand, there was a significant correlation in F_2 between pollen fertility and seed fertility. The coefficient $r = 0.9306$ when

$n=103$. This seems to indicate that the same factors were responsible for the drop of both pollen fertility as well as seed fertility.

A. chrysantha had more ovules in its long follicles than were contained in the short follicles of *A. ecalcarata*. Moreover, *A. chrysantha* had much larger seeds. For this reason the dependence between these two traits in F_2 was calculated. The coefficient of correlation between the length of follicles and the size of seeds was $r=0.4658$ when $n=128$.

In the F_2 population of 128 individuals the coefficient of correlation between the follicle length and the ovule number was $r=0.5239$. The coefficient of correlation between the number of ovules and seed size was $r=0.4324$ when $n=128$.

DISCUSSION OF RESULTS

The hybrids obtained in this investigation between *Aquilegia ecalcarata* and *A. chrysantha* have once again confirmed the opinion that in the genus *Aquilegia* interspecific crosses even between distantly related species and the progeny from such crosses can be obtained without major difficulties (Anderson and Schafer 1931). In F_1 of the hybrids obtained in the course of this investigation seed fertility was strongly and pollen fertility somewhat reduced, but at the same time in meiosis there were no visible symptoms of disturbances. Moreover, a large proportion of seeds did not germinate and a part of the seedlings perished at an early stage. The zygotic sterility may result from the meeting and the mutual interaction of unharmonious sets of genetic factors. The same explanation is suggested by Skalińska (1928) for the sterility of some hybrids of *A. chrysantha* with various species from the *vulgaris* complex that she experimented with in her investigations. In spite of the elimination of a part of micro- and macrospores and of some zygotes in various stages of development a regular segregation of most of the investigated traits was observed in the second generation of the cross between *A. ecalcarata* and *A. chrysantha*. Such traits as the height of plants, the shape of leaves, the size of petals and sepals, the length of follicles, the number of ovules in follicles, the size of seeds, and pollen and seed fertility all manifested a continuous variation, which seems to indicate that in the case of these traits many factors were segregated.

The absence or presence of spurs, the growth habit of flowers, and the colour of flowers were controlled by a different hereditary mechanism. The results reported in this author's preceding paper (1960) led to the conclusion that in *A. vulgaris* only one gene was responsible for the formation of spurs. In the present experiments it was found that the absence of the spur in *A. ecalcarata* was recessive with regard to the presence of the spur in *A. chrysantha*, since all the F_1 generation had

spurs. On the other hand, the segregation in F_2 according to the ratio 15 : 1 (15 with : 1 without spurs) justifies the assumption that the formation of the spur in *A. chrysantha* was determined by two dominant genes. The question remains whether this genetic composition occurs in all the American *Aquilegia* species, or is characteristic only for *A. chrysantha* or the species from the *caerulea* complex.

The difference between the inheritance of the spur length in F_2 of the cross *A. ecalcarata* $\times$ *A. chrysantha* and in F_2 of the cross *A. vulgaris* $\times$ *A. ecalcarata* is very interesting. Although in the latter instance 3/4 of individuals with spurs manifested the normal polygenic segregation reflected by the spur-length variation curve of the usual shape, in the former instance 15/16 of individuals with spurs were divided in almost equal numbers between the particular spur-length classes (2 to 23 mm.). This means that in each case the inheritance of the spur length was controlled by a different mechanism. For the understanding of the still unexplained mechanism underlying the inheritance of the spur length in the cross *A. ecalcarata* $\times$ *A. chrysantha* more experiments on the further generations and backcrosses are necessary.

If it is true that in the genus *Aquilegia* the morphology of flowers reflects adaptations to different animal pollinating agents (Grant 1952), then the growth habit and the colour of flowers must also have a great significance. The yellow colour and the erect position of flowers in *A. chrysantha* favour the pollination by the sphinx-moths, because the yellow colour is better visible at night. The information contained in Munz's monography (1946) seems to indicate that the type with drooping flowers predominates among the Eurasian species which are pollinated by flies and various bees. The American species of the *caerulea* complex pollinated by moths have erect flowers. The segregation in F_2 of the cross *A. ecalcarata* $\times$ *A. chrysantha* according to the ratio 3 : 1 (3 drooping : 1 erect) leads to the supposition that the transition from the drooping to the erect growth habit was accomplished in one mutation stage. The trait of the drooping flower was dominant with regard to the trait of the erect flower.

On the ground of investigations on the heredity of flower colour in diploid crosses between *A. flabellata nana* and *A. chrysantha* Skalińska (1935) assumed in *A. chrysantha* the basic factor *C* responsible for the occurrence of colour in flowers and the factor *Y* responsible for the yellow colour. In an earlier paper on the reciprocal crosses between *A. vulgaris* and *A. chrysantha* Skalińska (1928) had assumed the presence in *A. vulgaris* of the factor *R* responsible for the pink colour of the cell sap and of the factor *F* modifying the pink colour to violet.

Analysing the inheritance of the flower colour in the crosses between

A. ecalcarata and *A. chrysantha* this author has made the following assumptions:

A. chrysantha introduced into the cross the factor *C* responsible for the occurrence of colour and

the factor *Y* causing the yellow colour of flowers.

The factors *R* and *F* present according to Skalińska in *A. vulgaris* have been here attributed to *A. ecalcarata*, however, with the reservation that the factor *F* modified the pink colour of the cell sap to purple and that *A. ecalcarata* also introduced the factor *C* into the cross. Then

the genotype of *A. ecalcarata* might have been *CCyyRRFF*, and

the genotype of *A. chrysantha* might have been *CCYYrrff*.

The first hybrid generation with two-coloured flowers, i.e. with both anthocyanin and yellow chromoplasts, could comply with the general formula *CCYyRrFf*.

The intensity and the distribution of colours on the surface of sepals and petals in F_2 can be disregarded, since these traits were undoubtedly controlled by other segregating genes; then the following basic types of flower colours could be distinguished:

a) Plants with distinctly two-coloured flowers having both the yellow pigment and anthocyanin. The colour shade of these flowers probably depended on the homozygous or heterozygous state of the factors *Y*, *R*, and *F* and could be e.g. purple-violet, violet-purple, dark purple, or light purple. Generally speaking the genotype of these plants might have been *CCY(y)R(r)F(f)*. There were 127 plants of this type, whereas theoretically they should have constituted 27/64 of the whole population i.e. 116 plants.

b) Plants with yellow flowers (of various shades of yellow). The plants in this group had not the factor *R*, and they either had or had not the factors *Y* and *F*. This means that the plants doubly homozygous with regard to the recessive genes *y* and *r* and having the genotype *CCyyrr* were not white but pale creamy regardless of whether they had or had not the factor *F*. The number of plants with this phenotype was 12.

On the whole, owing to the absence of the factor *R* the plants in this group had no anthocyanin in the flowers or only had traces of this pigment. The total number of the plants in this group was 58 (12 + 46). Theoretically they should have constituted 16/64 of the population, i.e. their number should have been 68.7.

c) Plants with pink or yellow-pink flowers and without the factor *F*. The general genotype of these plants was *CCY(y)R(r)ff* and their number was 56. Theoretically they should have constituted 12/64 of the whole F_2 population, and thus their number should have been 51.6.

d) Plants with purple or violet-purple flowers and no yellow pigment. The general formula of these plants probably was *CCyyR(r)F(f)*. There

were 34 such plants. The theoretical proportion of this group in the F_2 population should have been $9/64$ i.e. 38.7 individuals.

As is to be seen the numerical ratio in the second hybrid generation was $127 : 58 : 56 : 34$, which agreed well with the theoretical ratio $27 : 16 : 12 : 9$. This justifies the assumption that there were three pairs of alleles (Y/y , R/r , F/f) responsible for the colour of flowers in the hybrid in addition to the basic factor C controlling the occurrence of colours in flowers and introduced into the cross by both *A. ecalcarata* and *A. chrysantha*.

The X^2 test showed that for $X^2=3.844$ (for three degrees of freedom) the value of p was more than 0.25.

The hybrids between *A. vulgaris* and *A. chrysantha* investigated by Skalińska (1928) were matroclinal and in F_2 there were no plants of the male-parent type. Neither were there any plants with two-coloured violet and yellow flowers. This means that in those hybrids the segregation was not typical. In the case of this investigation a similar effect was not observed and, in spite of the rather strongly reduced fertility and the high zygotic sterility, the segregation was normal.

The genetic analysis of the hybrids between *A. ecalcarata* and *A. chrysantha* clearly indicates that three out of the 14 investigated traits distinguishing these two "distant" species were inherited according to the Mendelian laws. The trait of the absence of the spur was probably controlled by two recessive genes and the spur developed even when only one dominant gene was present. The drooping or erect growth habit of flowers depended on one basic gene, whereas the colour of flowers was controlled by relatively few factors which segregated independently of each other but co-operated in their phenotypic manifestations. The other specific traits were controlled by the action of multiple genes which were not identified owing to their slight influence on the phenotype.

SUMMARY

1. The inheritance of specific traits in hybrids of *Aquilegia ecalcarata* with *Aquilegia chrysantha* was studied.

2. The haploid number of chromosomes in both species was $n = 7$ and meiosis in the first hybrid generation proceeded without visible disturbances.

3. In the first hybrid generation both the pollen fertility and the seed fertility was reduced; the drop in the latter case being considerable. In F_1 pollen fertility was 62 per cent and seed fertility 8 per cent.

4. In the second generation of the cross *A. ecalcarata* $\times$ *A. chrysantha* the segregation of the following traits was continuous: the height of plants, the shape of leaves, the size of petals and sepals, the length of follicles, the number of ovules in follicles, the size of seeds, and pollen and seed fertility. The variation of these traits in F_2 was continuous which seemed to indicate that many polymeric factors were being segregated.

5. The absence of the spur in *A. ecalcarata* was recessive with regard to the presence of the spur in *A. chrysantha*. The whole F_1 generation was uniform in that it had spurs of intermediate length. In the second hybrid generation 1/16 of the total number of individuals had flowers without spurs and 15/16 of individuals had flowers with spurs. This means that the trait of the absence of spurs could have been controlled by the presence of two recessive genes. For the development of spurs only one dominant gene was necessary.

6. The trait of the erect flower in *A. chrysantha* was recessive with regard to the trait of the drooping flower in *A. ecalcarata*. The segregation in F_2 was according to the ratio 3 : 1 (for three plants with drooping flowers there was one with erect flowers). This means that this trait was controlled monogenically.

7. The colour of flowers in the F_1 hybrid generation was determined by the concurrence of the following factors:

factor C determining the presence of a colour,

factor Y determining the yellow colour (both these factors were introduced by *A. chrysantha*),

factor R determining the pink colour of the cell sap, and

factor F modifying the pink colour to purple (the last two factors as well as factor C were introduced by *A. ecalcarata*).

The first hybrid generation was uniform in that it had all the flowers of two colours: purple and yellow. As the result of the independent segregation of the factors listed above there were in the second hybrid generation four kinds of plants: 1) with two-coloured flowers (resembling F_1), 2) with yellow or creamy flowers, 3) with pink or pink-yellow flowers, and 4) with purple flowers. The mutual proportions between the numbers of plants with the different kinds of flowers approached the ratio 27 : 16 : 12 : 9.

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Observation on the regeneration of the plasmodium of the Myxomycete *Didymium xanthopus* (Ditm.) Fr.

L. RAKOCZY

1. INTRODUCTION

A remarkable regeneration capacity is characteristic of myxomycetes in the plasmodial stage. It is connected most probably not only with the primitive character of this group of organisms but with the fact as well that the plasmodia unprotected by any membrane are often exposed, under natural conditions, to injuring agents.

In laboratory cultures this regeneration capacity greatly facilitates the multiplication of plasmodia by transferring their fragments on a fresh substrate. A detailed study of the regeneration processes following a mechanical injury of the plasmodium presents not only a scientific interest, but may be of some importance for laboratory practice. In spite of numerous studies concerned with the physiology of myxomycetes little is known however about their regeneration capacity. Besides occasional observations a study on regeneration was made by Skupieński (1953) who described the macroscopical changes occurring in injured plasmodia of *Didymium squamulosum* Fries.

The study reported in this paper was undertaken with the aim of examining the microscopical changes following the injury of the plasmodia of *Didymium xanthopus* and those occurring during the course of regeneration. The study was completed by an investigation on the dependence of the regeneration ability on the age of the culture and the local differentiation of the plasmodium.

2. METHOD

The experimental material, the myxomycete *Didymium xanthopus*, was collected in the form of a sporangium found on a old rotten pine trunk in July 1958 in a small wood in the vicinity of Busko.

Plasmodia were obtained by sowing out the spores on a 2,5% carrot extract solidified with 1,5% agar; 3 weeks after the spores has been sown out lemon yellow coloured plasmodia appeared. They were subsequently cultured on oat agar prepared after Howard (1931).

Cultures were maintained in vegetative state by inoculating them successively (every 6—7 days) on the above mentioned nutrient medium in test tubes kept in a dark thermostate at 20°C.

The material for experiments was obtained by transferring fragments of the plasmodium from the stock culture (from oat agar) on a washed nonnutritive agar on Petri plates. Observations were performed 1, 2, 3, 5, 10 days following inoculation directly on the plates. A Lumipan microscope equipped with a condensor for small magnifications and an objective (10 × or 5 ×) was used.

The plasmodium was cut by means of an arrangement shown in fig. 1. A narrow piece of a razor blade was fastened in a ring attached to the objective; the blade was situated about 3 mm. below the lens of the

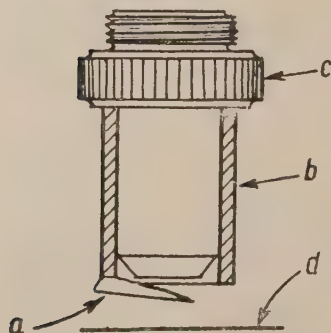


Fig. 1. Arrangement for cutting plasmodium threads: *a* — piece of razor blade; *b* — fastening ring; *c* — microscopical objective 10 ×, *d* — plane of object

objective and was not visible in the microscopic field. A swift lowering of the microscopic tube permitted to make a straight cut through a plasmodial vein in a desired place. A rapid lifting of the microscopic tube made possible the observations of the cut fragment as early as 1 sec. after operation.

An apparatus described by Zurzycki (1958) was used for registering the speed of the streaming protoplasm.

Fragments of veins and of the frontal part of plasmodia provided the material for investigations on regeneration capacity of small isolated fragments. These fragments were previously immersed in water for 5—10 minutes in order to clean them from the agar substratum. The fragments were then transported on fresh agar in a Petri plate on which they were directly observed.

The migration and growth of the plasmodia developed from fragments of different age and size were observed in Rayan's tubes filled with agar medium (oat agar). The tubes, 1.4 cm. in diameter, were 30 cm. long. The plasmodial fragment was introduced into one end of the tube and the successive positions of frontal part of the plasmodium were read every day in 24 hours intervals. Each series is the mean of 20 determinations.

3. RESULTS

The course of regeneration

Even a slight contact of a plasmodial vein with a razor blade, without cutting it, caused an immediate inhibition of the streaming of protoplasm on the length of 0.5—1.5 mm. extending to both sides from the place of contact (Plate I, fig. 5—8). A local thickening, of the vein in this place was visible for a few seconds following the mechanical stimulation (Plate I, fig. 6). The zone of inhibited motion decreases after 10—30 sec.; a local inhibition, however, in the place the stimulus was applied remains still for some more minutes. Finally the influx of protoplasm produces an increase of breadth and thickness of the vein in the place the operation was performed (Plate I, fig. 8). After 3—5 minutes the blocked vein becomes again accessible to protoplasmic streaming. Initially the channel of streaming protoplasm is narrow in the place of application of the stimulus and for this reason the motion rate of protoplasmic granules locally increases. The thickening of the cortical layer of protoplasm disappears after some more minutes and the vein regains its normal appearance (Plate I, fig. 8).

A complete cut made through a vein with a sharp blade leaving both fragments in the very place causes a cycle of phenomena presenting some differences according to the thickness of the cut vein, its physiological state and the age of plasmodium. The observed phenomena may be classified into 3 types:

Type I. Outflow of protoplasm is characteristic of this type. It occurs only after cutting thick veins (200—300 μ in diameter) of a normal plasmodium. The cut is followed by an immediate outflow of endoplasm. Probably the mechanical stimulus is capable of causing the gelatinisation of the whole protoplasm in a large vein in a short time and for this reason the inner pressure in the vein presses out the liquid endoplasm (comp. photos 9—12, plate I). After removing the blade the two fragments of the cut vein join again and the effused protoplasm causes the formation of large globular blisters of about 1 mm. in diameter. About 1 sec. after the cutting operation the shape of the effused protoplasm is final and is the result of the solidification of its peripheric part (Plate I, fig. 10). Protoplasmic streaming in the vein is inhibited within 1—1.5 mm. distance extending to both sides of the injured place. Within 2—5 sec. following operation a contraction of the effused protoplasm takes place and lasts for about 20 minutes (Plate I, fig. 11). Subsequently the effusion maintains its shape and dimensions unchanged for a longer time. The motion of protoplasm in the more removed parts of the plasmodium causes an increase of the rate of protoplasmic streaming in side veins. It may also

produce their thickening. Sometimes a secondary break of the ectoplasmatic layer is observed as a consequence of the pressure exerted by the streaming protoplasm. Sometimes a secondary break of the first protoplasmic outflow and an increase of its size following injury were also observed. Finally in few cases a new effusion is formed in the distance of about 1 mm. from the injured place within 30—60 sec. (fig. 2). In the course of time the zone of inhibited motion shrinks and after some minutes

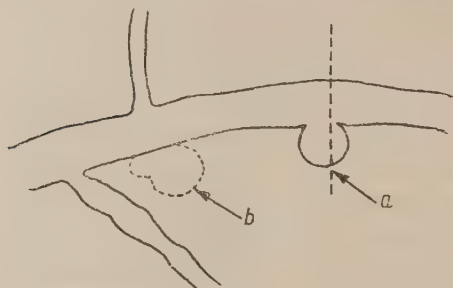


Fig. 2. Secondary effusion of protoplasm: *a* — plane of cutting with primary effusion; *b* — secondary effusion appeared 30 sec. after operation

the communication in the vein is restored. In this period a gradual decrease of the size of the effusion and its resorption are observed (Plate I, fig. 12). After 0.5—1.0 hour the shape of the effusion in the injured place does not much differ from normal protrusions occurring in other places of the vein.

Type II is characterised by a gelatisation and contraction of the fragments of the cut vein followed by regeneration processes resulting in a fusion between the two fragments. Processes of this type occur most frequently either in veins of average size or in small ones in young plasmodia whereas in old plasmodia they are observed in thick veins and as a rule are characteristic of cuts made in the frontal part of the plasmodium.

The course of processes occurring after cutting a vein can be seen from fig. 13—19 (Plate II); a sudden inhibition of protoplasmic streaming takes place immediately after injury. It extends to the length of 2 mm. to both sides from the place of operation. If there is a side vein in the cut fragment the inhibition of motion spreads as far as the side vein, but it also may extend over it. A contraction of both the cut fragments and the formation of a small fissure between them are visible already 1 sec. after injury. This contraction increases within the following 5—15 sec. and results in an enlargement of the fissure the size of which may attain even $150\ \mu$ (as a rule $50\text{--}100\ \mu$) (Plate II, fig. 15). Within the following 20—30 sec. the zone of inhibited motion begins to shrink. Unless the protoplasm streaming from more remote parts of the plasmodium finds an outlet in side veins, a considerable mass of protoplasm is accumulated

in the injured place. This accumulation leads to a thickening of the corresponding vein (or veins) and results in a rounding up of their outlines. The accumulated protoplasm is not removed even in the periods of reverse streaming, in contrast to type III.

The protoplasmic streaming starting in the zone of inhibited motion after about 10—20 sec. (sometimes even earlier) is not a mass transport of protoplasm normally observed in an injured vein. Initially it is of local character, restricted to short distances and occurs irregularly at a low rate and is, as a rule, interrupted by longer periods of stagnation (Fig. 3).

It is only in the later periods that the streaming becomes more coordinate, extends over longer distances and becomes rhythmical. Its rate, however, is still much lower than the rate of streaming shown by a normal vein. Due to this streaming, however, larger quantities of

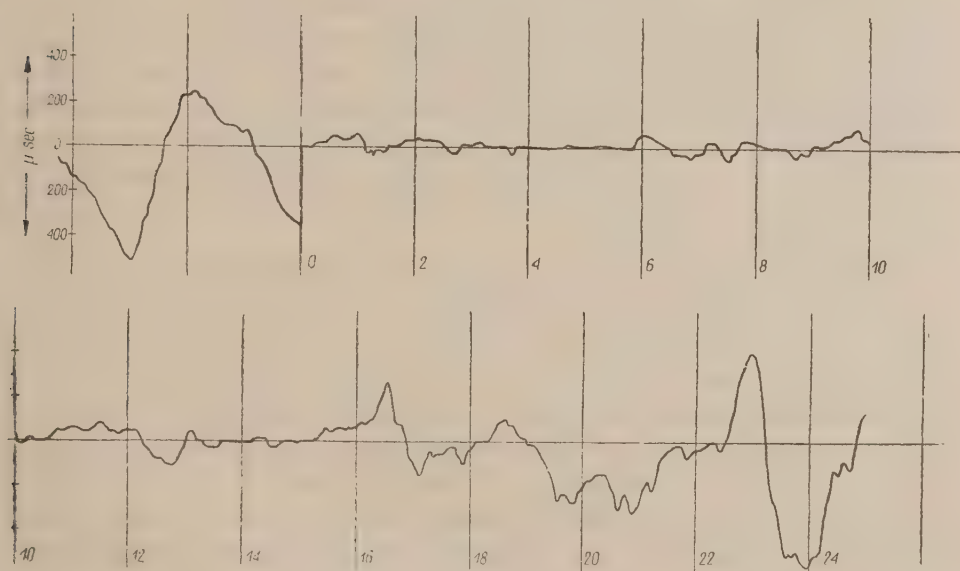


Fig. 3. Speed of protoplasmic streaming in the operated thread: X-axis — time in minutes (0 — time of operation), Y-axis—speed of protoplasmic streaming in μ/sec .

The record was taken at a point distant 300μ from the plane of cutting

protoplasm are gradually transported to the injured place; the result is a formation of lobes of protoplasm. The two parts of the cut vein approach each other and eventually they join after 5—15 minutes, depending on the intensity of the protoplasmic streaming (Plate II, fig. 17). In the place of junction the cortical layer of the vein is relatively thick and remains thick for a long time. Finally a channel is formed by means of which endoplasm may flow again in the veins.

Initially some disturbances in streaming may occur if the rhythms in the both fused fragments are not concordant as to their phases. Within few minutes the protoplasmic streaming regains its regular character and the motion rate which was low before the restoration of communication in the veins increases in later period considerably (Fig. 3).

The place where the injury was performed is visible on the vein for a long time in the form of an accumulation of protoplasm (Plate II, fig. 18) or an inflection in the vein caused by an unsymmetrical fusion of the two fragments. Also the optical properties (light refraction) are modified in the place of junction. In plasmodia showing strong vitality (young plasmodia on a humid substratum) the accumulation of plasma in the place of injury initiates the formation of a new vein with side veins extending along the line of cutting on the agar substratum.

If the vein is cut without injuring the agar substratum and one of the two fragments is removed, the processes occurring in the left fragment are similar to the above described (Plate II, fig. 20 and 24). They consist in the liquefaction and expansion of protoplasm in the cut place and formation of protoplasmic lobes, the development of which depends on the local physiological state of the plasmodium. If the plasmodium tends to form frontal parts, the lobes may give rise to a new local front; otherwise the normal sequence of events is their withdrawal followed by a gradual reduction of the cut fragment.

Similar processes occur after injury of a healthy frontal part of a plasmodium (Plate III, fig. 25 and 30). The cut causes an immediate inhibition of motion in the veins, whereas local streaming in the thin lobes of protoplasm joining the veins may continue. The contraction of the cut fragments results in the formation of a fissure (100—150 μ) separating the fragments for several seconds (Plate III, fig. 26). Subsequently the motion in the veins is gradually restored and leads to a characteristic accumulation and withdrawal of protoplasm from the fragments in the injured place. The motion rate in the thin frontal veins decreases and the protoplasm tends to aggregate (Plate III, fig. 27) into small droplets which, however, spread again after some minutes and form a delicate network of small veins. The wound edges of the two vein parts gradually cicatrize, their outlines become irregular and after a time they fuse (Plate III, fig. 28). Fusion takes place first between the principal veins and subsequently along the whole length of the cut. Protoplasm is accumulated along the line of the cut and this accumulation leads to the formation of a new vein during the course of further expansion. From this accumulated protoplasm starts the spreading of thin lobes of protoplasm.

Type III. This type of regeneration is not a regeneration in the exact meaning of this term — because it leads finally to a disparition of the injured vein (Plate III, fig. 31 and 36). It is observed only in young

plasmodia in small anastomosing veins, whereas in older or less healthy ones it may also appear in thicker veins, sometimes also in the frontal part. The cut is immediately followed by an inhibition of motion in the vicinity of the operated place and a contraction of the cut fragments. In contrast to the previously described type where the contraction is transient and attains its highest degree after 10—15 sec., in this type the contraction is permanent and increases with time (Plate III, fig. 32 and 33). The protoplasmic streaming which gradually appears in the zone where the motion was inhibited causes a withdrawal of protoplasm from the injured place. Only exceptionally a greater accumulation in the cut fragment may temporarily appear. The plasma does not accumulate in the wounded place and at the moment of maximal withdrawal of endoplasm from this place a further contraction of the injured part of the cut vein is observed. It is manifested by the shortening of the vein fragments. Their contraction proceeds at an irregular rhythm by fits and starts. The contraction period lasts 1—3 sec. and is followed by a period of stagnation. This may be caused by the resistance resulting from the adhesion of the plasmodium to the substratum. After some minutes a complete liquidation of the vein is observed and the protoplasm is completely withdrawn from the injured vein (Plate III, fig. 36).

The influence of potassium oxalate solution on the regenerative processes

The necessity of calcium ions for the regeneration of injured protoplasm is widely known. Their elimination from the environment causes an inhibition of surface precipitation reaction which is a necessary condition of cicatrization of the wound (Heilbrunn 1956).

Using 0.1 M solution of calcium oxalate as a substance bounding the calcium ions observations were made on the role of calcium ions in the process of regeneration of the plasmodium.

The flooding of the plasmodium cultured on agar with the above mentioned solution causes its decay in a short time. The first and immediate effect is an inhibition of protoplasmic streaming in the veins. In some cases it occurs in a fraction of a second, in other cases (in thicker veins as a rule) it takes some seconds to occur. After 10—20 sec. the protoplasmic streaming is restored (in the same direction as before the inhibition); but soon it becomes irregular. A simultaneous contraction of the gel layer in the veins is observed. It results in an increase of pressure of endoplasm that causes in turn splitting of this layer and formation of outflows of protoplasm. Globular outflows 1 mm. in diameter are visible even with a naked eye. The first ones appear about 60 sec. following

application of the potassium oxalate solution. In contrast to the previously described effusions resulting from mechanical injuries of veins, outflows of protoplasm in this case do not show any distinct connection with protoplasmic streaming. Surface vacuolization is observed in numerous parts of the plasmodium, especially in the central ones. Hyaline vesicles appear on the surface of smaller and bigger veins as well as on the surface of the outflows. After 2—3 minutes a typical granulation of protoplasm takes place. The yellow pigments washed out from the protoplasm form stains on the agar. After 4—6 minutes ragged fragments of veins and colourless granules of protoplasm are the only remnants of the intact plasmodium.

Since the immersion of the whole plasmodium in a Ca ion bounding environment causes its complete degeneration in a very short time, an attempt was made to examine the influence on regeneration of locally administered potassium oxalate. The operation consisting in cutting the plasmodium vein (as formerly described) with the modification, however, that the blade was moistened with 0.1 M potassium oxalate solution. The course of regeneration in this case differs somewhat from that previously described (Plate IV, fig. 37—44). An inhibition of protoplasmic streaming on a certain length and a contraction of the cut vein fragments are observed; the very place of operation, however, is undistinctly outlined, shapeless (Plate IV, fig. 37 and 38) and after 1—2 minutes a distinct layer separating the healthy protoplasm from the injured is formed (Plate IV, fig. 39 and 40). A similar wound barrier is described by Seifriz (1955).

In places where the vein was blocked by a wound barrier accumulation of protoplasm is observed. From these places on the expansion of the plasmodium begins (Plate IV, fig. 42). The granular remnants of protoplasm dispersed among the centres of expansion are not involved in the process of regeneration.

Regeneration of the plasmodium from small fragments

Small fragments of veins from the frontal part of the plasmodium were amputated by means of a razor blade in order to examine the regeneration capacity of a new plasmodium from isolated fragments. These fragments were transported on pure 1.5% agar.

In all cases, the regeneration process is as a rule similar to the above described (comp. photos, plate V). The vein or lobe fragment cut off from the frontal part of the plasmodium is in the gel state and in this form it is transferred to a new substratum. After 15 minutes a certain rounding of its outlines and pulling in of plasmatic threads is observed. This process is doubtlessly connected with a partial liquefaction and contraction of

protoplasm. After 15—60 minutes thin frontal lobes begin to expand (Plate IV, fig. 41; Plate V, fig. 51). With very small fragments they spread equally in all directions; the fragments form slowly a new network of veins which initiates a new round or elliptic plasmodium (Plate V, fig. 49 and 52). It is only when the fragment of the frontal part is considerably greater that the newly formed plasmodium is fanshaped and has a clearly distinct frontal part. When a fragment of the frontal part or of a small or averagely thick vein regenerates, the accumulated protoplasm disappears earlier than when a fragment of a thicker vein starts the regeneration process. After 1—3 hours the transferred fragment is no more visible but is transformed into a new plasmodium with distinct frontal and distal parts which begins to migrate on the substratum (Plate V, fig. 53 and 54).

When compared with regeneration of cut veins the regeneration of small fragments is characterized by a complete lack of protoplasmic streaming in the amputated fragment. The change from gel into sol state is accompanied by a slow spreading of protoplasm over the substratum and by formation of thin lobes and veins around the transferred fragment. The size of the isolated fragment is of no importance for the speed of formation of a new plasmodium. Three regenerating fragments, one about 0.2 mg. and two much smaller, under 0.01 mg., are shown in photos (Plate V, fig. 45 and 49). The fresh weights of these fragments were estimated from their dimensions, the weight of fragments taken from similar veins having been determined previously by means of a semi-microanalytic balance. The three fragments begin to form protoplasmic lobes almost simultaneously. The only difference is that in greater fragments the primary accumulation of protoplasm is visible for a longer time, whereas, in very small ones it disappears completely in about 30 minutes.

When comparing the regeneration of fragments taken from the frontal part with that of fragments taken from distal veins of the same plasmodium no essential differences in the course of regeneration are observed (Plate V). Some differences, however, especially in the initial period could be observed depending on the age of the plasmodium the fragments were taken from.

In regenerating fragments taken from old plasmodia protoplasmic lobes appear a little earlier than in fragments from young plasmodia.

The above described course of regeneration is observed only if the plasmodial fragments are transferred on fresh agar in the way mentioned in section 2, i.e. with no trace of the old substratum adhering to the fragments. Fragments of old cultures, on the other hand, transferred with a layer of agar on which they developed (as it is generally practised) show a poor migration ability on fresh agar and often perish.

Influence of age and size of the implant on the growth and migration of the plasmodium

In connexion with the fact that the regeneration capacity depends on the size and the age of the fragments, there arose the question of what size and age implants should be used to obtain cultures of uniform growth.

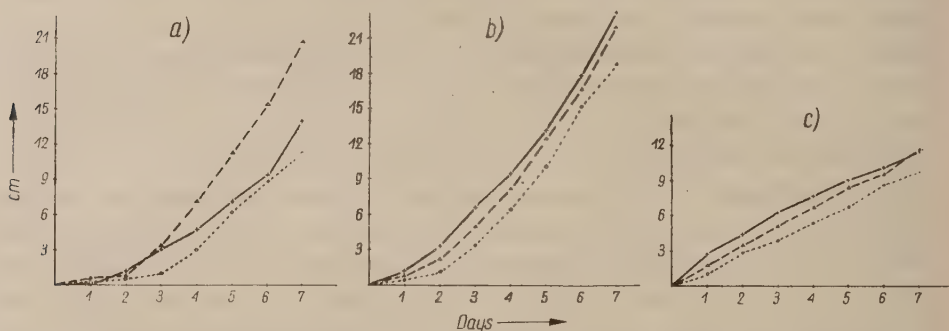


Fig. 4. Growth and migration of plasmodium in Rayan's tubes. Fragments of the frontal part used for inoculation were taken from 4 days (a), 8 days (b), 14 days (c) old cultures. The sizes of fragments were about 10 mm² , 40 mm² — — — , 150 mm² —————, X-axis — time in days, Y-axis — distance in cm

The method of culturing plasmodial fragments on oat agar in Rayan's tubes was applied for investigations on this problem (Rayan, Beadle and Tatum 1943). The cultures were maintained in the dark at 20°. In these conditions the plasmodia may be cultured for 20 days.

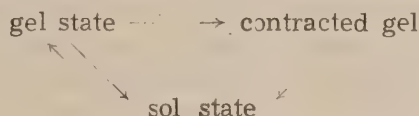
Fragments taken from the frontal parts of plasmodia of about 10, 40, 150 mm² area, together with a thin layer of culture agar were transferred to one end of the Rayan's tubes. The positions of the moving fronts of the plasmodia were read daily. Results are given in fig. 4, a—c. Compared with fungi the migration of plasmodia shows less regularity. This can be explained by the fact that the successive positions of the plasmodia are the result of their migration and growth (sometimes it happens that the plasmodium temporarily migrates in opposite direction). It results from the graphs that the most regular growth and migration are observed with 8 days old plasmodia and are only to a small extent dependent on the size of the fragments used for transplantation. With older material the migration of the plasmodium is much slower; with younger material — a delayed stabilisation of the migration rate and an obvious influence of the size of the implant are discernible. It must be stressed that plasmodia grown on oat agar in small Petri dishes provided the stock material for these observations. This is a detail that is important from the methodical

view point, because 8 days old cultures are at the climax of their development. In these conditions the size of the inoculum is without influence on rate of growth and migration of the plasmodium provided the growth rate has attained its constant value (after about 2 days).

DISCUSSION

In all cases, a sudden cut of the plasmodium caused an immediate inhibition of protoplasmic streaming along a certain length of the injured vein and its gelatination. This phenomenon is a known reaction to a shock stimulus and was many times described (Winer and Moore 1941, Seifriz and Erstein 1941 and others).

In all cases the cut is followed by a more or less intensive contraction of the two fragments. Before their subsequent expansion a part of protoplasm at least becomes liquid, this being shown by sporadically occurring protoplasmic streaming. The observed phenomena may be compared with the scheme proposed by Landau (1959) to explain the changes of sol into gel state occurring in amoebas. According to this scheme the expansion of the contracted gel may only occur by passing through the sol state.



The temporary and local protoplasmic streaming discernible in the zone of inhibited motion presents some interest from the point of view of mechanics of motion. These streamings are not a mass transport of plasm but rather local streams which obviously are not synchronised either between themselves nor with the mean stream. It is only after a considerable length of time that their range enlarges and a coordination with the main stream takes place. This phenomenon presents some similitude with the beginning of the rotation movement in *Elodea cells*. It is always preceded by local streams of sliding type whose intensities increase till they change into a coordinate rotation movement.

According to Linsbauer (1929) a mechanical shock exerts a similar effect on the protoplasmic streaming in *Chara cells*.

In myxomycetes a local injury of ectoplasm causes an inhibition or a disturbance of motion in the place of injury, blocking of the vein (on a certain length), accumulation of protoplasm, local changes of the direction of streaming etc. As a rule no effusion of protoplasm from the veins is observed, this process being inhibited by an immediate gelatination. The cut of thick veins only, with a large endoplasmic channel is accompanied by an effusion of protoplasm which, however, rapidly (about 1 sec.) forms a gel membrane. This membrane prevents, a further effusion

of protoplasm. In contrast to *Nitella* (Strugger 1949, Kamiya 1959) this phenomenon is observed even after an immersion of the plasmodium in water.

If a plasmodium is placed in a medium capable of bounding calcium ions (0,1 M solution of potassium oxalate) the appearing symptoms are characteristic of a lack of surface precipitation, described by Heilbrunn (1956) and Strugger (1949). The veins undergo a spontaneous bursting accompanied by frequent effusions of protoplasm. However, a great amount of Ca ions present in the endoplasm interferes with the effusion and contributes to the formation of a new gel membrane on the surface of the effusing endoplasm. A far going destruction and a final decay of the plasmodium are the result of the action exerted by potassium oxalate.

When the injuring factor is applied locally, it affects only a small fragment which is separated from the rest of the vein by a wound barrier. In the noninjured parts the regenerative processes proceed in a normal way.

Our study has shown that fragments of plasmodia possess a remarkable regeneration ability. Even a very small fragment of about 0.01 mg. fresh weight has not lost its ability of reproducing a new plasmodium on a suitable substratum. Its further growth depends on the culture conditions and the chemical properties of the substratum.

The observed phenomena are in agreement with the results of investigations published by Skupieński (1953). Although a different species (*Didymium squamulosum*) was used in his investigations, the rates of the regenerative processes in both cases are nearly the same.

SUMMARY AND CONCLUSIONS

1. A detailed description is given of the processes following an injury made on a vein or the frontal part of plasmodia of the myxomycete *Didymium xanthopus*. The first effect is an inhibition of protoplasmic streaming in the plasmodial vein on a certain distance from the injured place. The effects of cutting a vein of the frontal part of the plasmodium into two fragments are their contraction and moving away from each other.

Regeneration processes may be classified into 3 types:

a) The first is characterised by an effusion of protoplasm which joins again the cut fragments. The effusion is followed by a restoration of protoplasmic streaming and a resorption of effused protoplasm.

b) In veins of average size or in frontal parts there is no plasmatic effusion after a cut. The two fragments contract and move off. After some time the streaming of protoplasm is restored. Plasma expansion in the

injured place leads to a fusion of the cut fragments and reestablishment of the former state.

c) In small anastomosing veins the cut fragments contract and move off. The restored movement of protoplasm and a further contraction lead to the resorption of the two fragments by the larger veins.

2. Disturbances of protoplasmic streaming observed after a performed cut of the vein and in the course of regeneration present some similarity to analogous processes occurring in higher plants.

3. A great regeneration ability of even very small fragments isolated from the plasmodium was established. This ability seems to be independent, in a wide range, on the size of fragments and on the place these fragments were taken from. To a certain extent it depends on the age of the plasmodium.

4. Treatment of the plasmodium with 0,1 M solution of potassium oxalate bounding Ca ions induces symptoms indicating the absence of a surface precipitation reaction in the protoplasm. The symptoms are similar to those observed in protozoa and in plants species with cell membranes. After 5—10 minutes the plasmodium becomes completely destructed and perishes.

5. The most regular growth and migration rate are shown by plasmodia whose inocula were taken from cultures in the period of optimal growth. For the examined species this condition is fulfilled by 8 days old cultures grown on oat agar.

I am indepted to Prof. Dr. F. Górski and Doc. Dr. J. Zurzycki for their kind advices during the course of the work.

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Explanation of the plates

Plate I

Figs. 5—8. — Changes following touching a middle size thread of plasmodium without cutting it. 5 — before operation, 6 — 2 sec. after operation. The arrow indicates the place of touching; 7 — after 35 sec.; 8 — after 5 minutes

Figs. 9—12. — Effusion of protoplasm after cutting a thick vein: 9 — before operation, 10 — 1 sec. after operation; 11 — after 5 sec.; 12 — after 25 min.

Plate II

Figs. 13—19. — Regeneration of the injured thread: 13 — before operation; 14 — 1 sec. after operation; 15 — after 1.5 min.; 16 — after 12 min., 17 — after 17.5 min.; 18 — after 25 min.; 19 — after 50 min.

Figs. 20—24. — Regeneration processes following the removing of a fragment of a cut thread: 20 — before operation; 21 — after 2.5 min.; 22 — after 35 min.; 23 — after 60 min.; 24 — after 75 min. Magnification as in Plate I

Plate III

Figs. 25—30. — Regeneration processes after cutting the frontal part of a plasmodium: 25 — before operation; 26 — 10 sec. after operation; 27 — after 5 min.; 28 — after 14 min.; 29 — after 22 min.; 30 — after 75 min.

Figs. 31—36. — Reduction of an injured thread.: 31 — before operation; 32 — 40 sec. after operation; 33 — after 4 min.; 34 — after 9 min.; 35 — after 15 min.; 36 — after 17 min. Magnification as in Plate I

Plate IV

Figs. 37—44. — Course of regeneration after cutting the thread with a razor blade moistened with 0.1 m potassium oxalate solution: 37 — 1 sec. after operation; 38 — after 20 sec.; 39 — after 3 min.; 40 — after 12 min.; 41 — after 15 min.; 42 — after 30 min.; 43 — after 1 hour; 44 — after 2 hours. Magnification as in Plate I

Plate V

Figs. 45—49. — Regeneration of plasmodium from small isolated fragments of a thread: 45 — immediately after transferring to a new substratum; 46 — after 30 min.; 47 — after 1 hour; 48 — after 1.5 hour; 49 — after 2.5 hour

Figs. 50—54. — Regeneration of plasmodium from a fragment of the frontal part of a plasmodium: 50 — immediately after transferring to a new substratum; 51 — after 1 hour; 52 — after 2 hours; 53 — after 2.5 hour; 54 — after 3.5 hour

Plate I

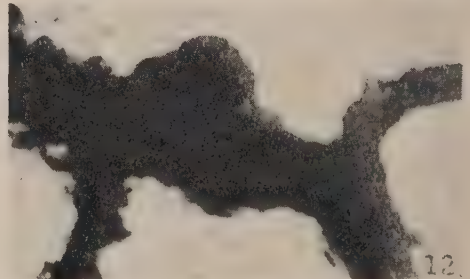
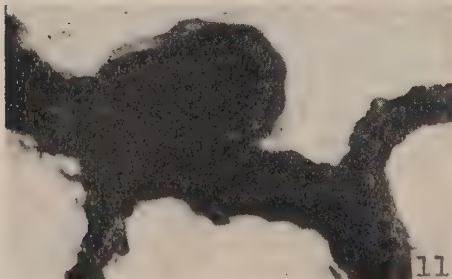
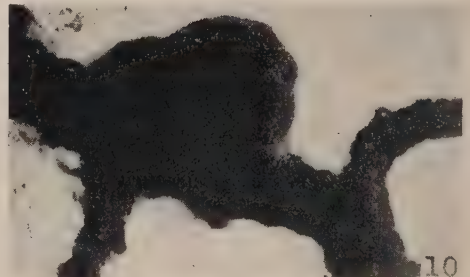
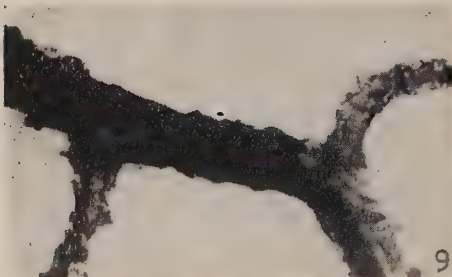
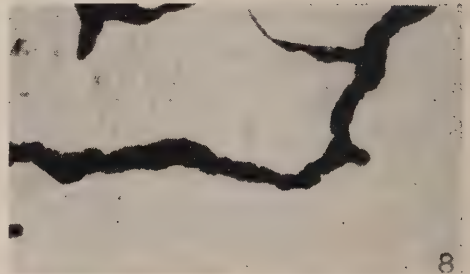
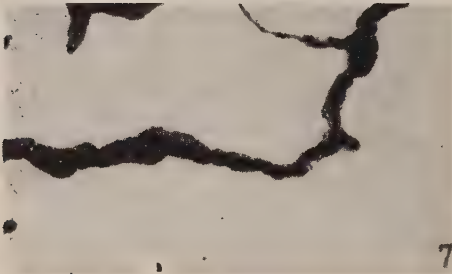
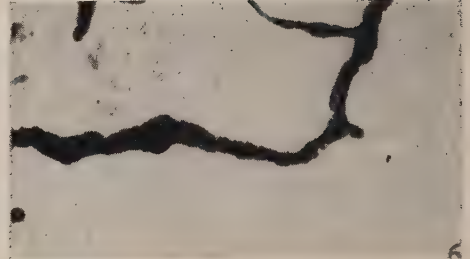


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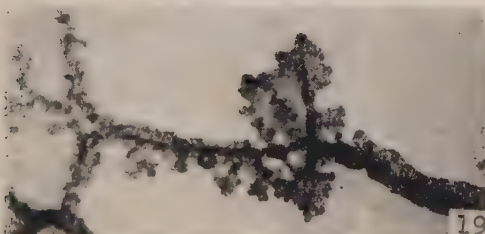
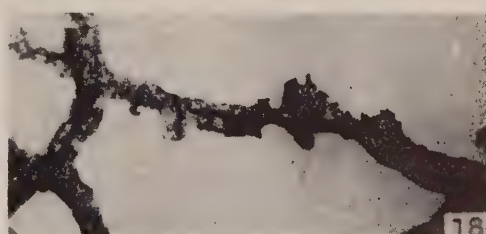
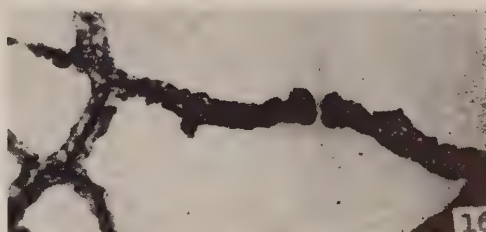


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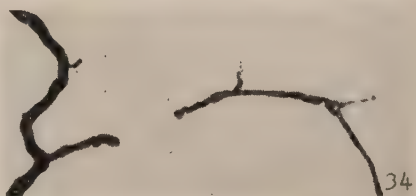
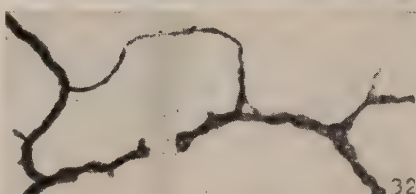
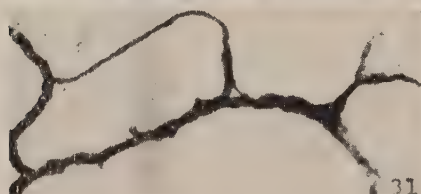
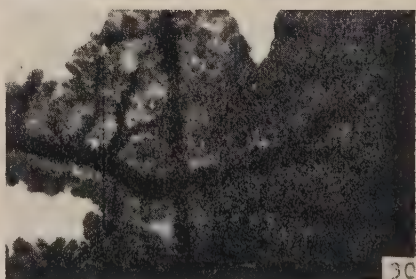
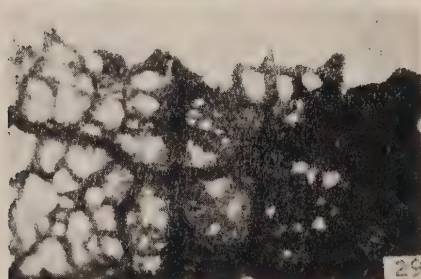
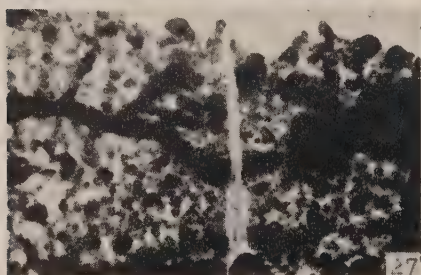
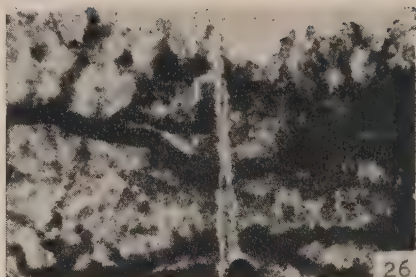
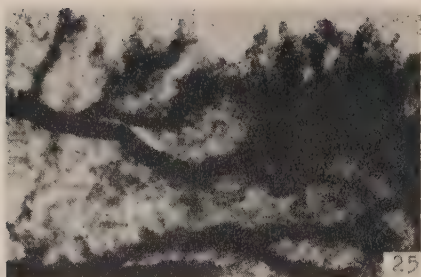


Plate IV

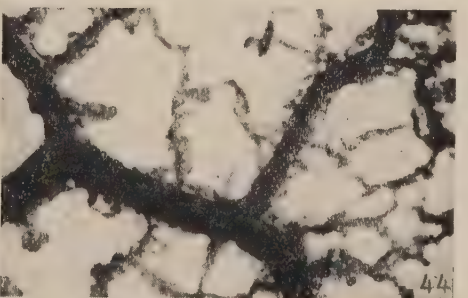
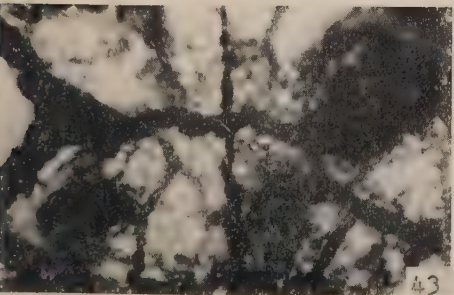
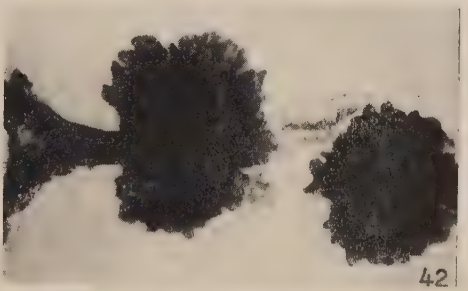
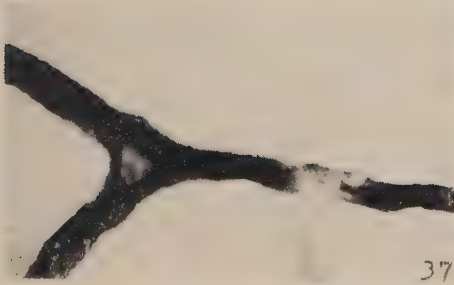
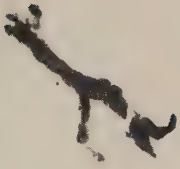
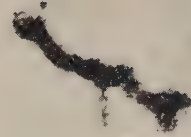


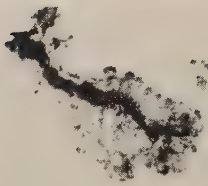
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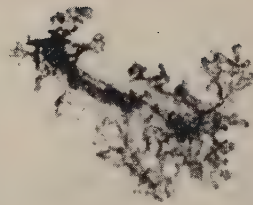
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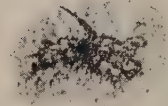
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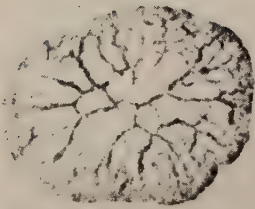
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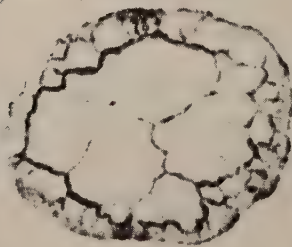
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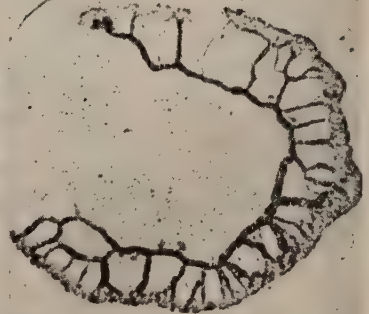
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Effects of the aqueous extract from *Poria obliqua* Bres. on the roots of *Allium cepa* L., *Vicia faba* L. and *Tradescantia zebrina* Loud

J. SZULETA

The various specific effects of the extracts from plants, used in popular medicine against cancer, on plant cells and especially on mitosis has been investigated in Department of Plant Anatomy and Cytology of the Warsaw University already from 1953. Since the preliminary tests indicated on the activity of some of these extracts it was assumed that the study of various fractions of these extracts on various test plants will enable to separate specially active substances.

In 1959 we started to investigate the influence of the aqueous extract from *Poria obliqua* Bres. (*Inonotus obliquus* Pers.) using as the test object roots of *Allium cepa*, *Vicia faba* and *Tradescantia zebrina* cultivated in tap water. Extractions from *Poria* were done in the cold or hot tap water. The most useful was the dried water extract obtained from Mgr. S. Piaskowski. Most often following solutions of the dried water extract were used: 0.2, 0.5, 0.7, 0.8, 1, 1.5 and 2 per cent. pH was that of the tap water used for solvent. To these solutions the test plants from water cultures were transferred for periods ranging from 2 hours to several days. After different periods of treatment with extract solutions the root tips were fixed or the whole plants were transferred again to water cultures and the root tips were fixed after 6, 12, 24, 48, 62 (or more) hours or they were kept for further culture and observations. The fixative used were acetoalcohol (1:3) or Navashin fixative modification (CrAF 0.5—1—20). The fixation time was 4 hours. Smear preparations were stained with acetoorcein or acetocarmin. The microtome sections were stained with gentianviolet. Feulgen's method was used both for smears and sections.

This is only a preliminary report of larger studies not completed.

The roots treated for 24 hours in room temperature by 1 per cent fresh solution of the extract and transferred to water show a little different colour of root cap and of the whole of root tip. After still longer period, from 24 to 36 hours, their colour changed still more (Plate I, fig. 1).

The cells of the elongation region lose their turgor and become flabby. These are symptoms of necrosis because after 3—4 days these parts of the roots usually fall off. Smaller concentrations of the extract as e.g. 0.5—0.7 per cent initially cause an inhibition and later a very strong retardation of the roots growth. With concentrations from 0.7 to 0.8 per cent after 3—4 days of very slow growth the root tips of the majority of *Allium*, *Vicia* and *Tradescantia* roots died out and the transfer to water is without effect. *Tradescantia* in water culture develops root hairs. However, when the roots are in 0.1—0.2 per cent extract solution for a period from 1 to 2 hours they lose the ability to develop root hairs. When after the transfer to the water the root continues its growth the root hairs are formed only on that part that was formed already in water (Plate I, fig. 2). Thus the formation and growth of root hairs was inhibited by the extract.

Morphologically the apical meristems together with root caps of roots treated by 1 per cent extract through 24 hours are badly damaged or destroyed and after the transfer to water they behave as decapitated ones. They form numerous lateral roots (usually just above the injured part) and often they regenerate the apical meristem and continue to elongate after several days. The different behaviour depends most probably on the different degrees and types of damage.

The toxic action of the fresh 1 per cent or stronger extract on roots meristematic cells was proved beyond doubt. After 24 hours of treatment with this extract no mitotic figures were found in treated root tips of *Allium*, *Vicia* and *Tradescantia*. Probably all mitoses were completed because newformed nuclei are observed. It may be also that a sporadic regression of newly started mitotic divisions takes place. After the transfer to water the cells from apical meristems never behave normally. In nuclei pycnotic at the beginning a chromatolysis is observed both in apical meristem and in root cap. The degeneration of the nuclei resembles that described by Tischler as karyorhexis and consists in fragmentation of the nuclei into small granules penetrating in cytoplasm or, more often, resembles ordinary karyolysis.

Very interesting are the effects of the treatment by 0.7—0.8 per cent extracts through 24—48 hours, or of 1.5 per cent extract in 3, 6—12 hours. In these conditions we observe a specific action of the extract on the chromatin and the chromosomes. In prophase with initially loose and distinct spiralization (Plate I, fig. 3) tape-like structures are formed connected by bridges (Plate I, fig. 4) or more or less rounded structures are formed which easily disintegrate in small granules or even disappear (Plate I, fig. 5).

In metaphase a very strong shortening of the chromosomes was stated.

Their length may be one half of the normal (Plate II, fig. 1 and 2) or they form spherical bodies (Plate II, fig. 4). At metaphase with shortened chromosomes the kinetic spindle is quite distinct and seems to be normal.

If other plants react in the same way this may be a very helpful treatment for long chromosomes counting.

In anaphase and telophase we observe also shortening of the chromosomes and often their vacuolation or even fragmentation (Plate III, fig. 3 and 4; Plate IV, fig. 1—5). In comparison with normal anaphase (Plate III, fig. 1) or telophase the chromosomes from the treated cells are shorter (Plate III, fig. 3) or even spherical (Plate IV, fig. 2), they often show constrictions (Plate III, fig. 4; Plate IV, fig. 1, 4 and 5) or fragmentate (Plate IV, fig. 1, 3, 4 and 5).

It was assumed that the vacuolation found in metaphasic, anaphasic or telophasic chromosomes (Plate II, fig. 5 and 6; Plate III, fig. 2 and 4; Plate IV, fig. 1, 4 and 5) may be due to the slight heating (up to 50—60°C) in acetoorcein smearing but control preparations with untreated plants showed no vacuolation of the chromosomes (Plate III, fig. 1).

The "vacuolation" of the anaphasic chromosomes results probably from the shrinkage of the chromosomes with simultaneous despiralization. The vacuolation is only spurious. Besides this, true vacuolation as a result of chromosomes degeneration does exist also.

Very striking is the shortening of the chromosomes up to spherical shape (Plate II, fig. 3 and 4; Plate IV, fig. 1, 2 and 4). It seems that to certain degree this phenomenon is reversible. It is possible that the shortening starts earlier for instance for the metaphase already at prophase and together with the shortening of the chromosomes also other reversible changes in the still living cell are connected. The removal of the active substance by washing out may cause the return of the chromosomes to their initial state if the whole cell is able to recovery. If the cell with shortened chromosomes died out and the extract is still acting then the chromatin destruction and chromatolysis enues or only chromatolysis without fragmentation is observed.

Reasuming our preliminary results on the action of water extract of *Poria obliqua* on the cells of *Allium cepa*, *Vicia faba* and *Tradescantia zebrina* we may state that:

1. The cells from apical meristems and root caps of the roots are specially sensitive to treatment with the extract.

2. The extract inhibits cell divisions probably specially through the action on the chromatin causing its final lysis.

3. Resting nuclei after initial period of pycnosis disintegrate in small droplets (karyorhexis) which is followed by karyolysis or without previous karyorhexis, karyolysis take place.

4. The chromosomes are shortened to spherical bodies and after prolonged action of the extract chromatolysis ensues.

5. Extract from *Poria obliqua* inhibits the elongation (growth) of cells. This may be seen from the damages in the region of cell elongation in *Allium cepa*, *Vicia faba* and *Tradescantia zebrina* roots and from the inhibition of the root hairs formation in *Tradescantia zebrina*.

Detailed presentation and discussion of the results obtained will be published later.

Department of Plant Anatomy and Cytology
University of Warsaw

(Entered: 30.3.1961 r.)

Explanation of the plates

Plate I

- Fig. 1. — *Allium* roots after 24 hours in 1% *Poria* extract and after 24 hours in water. Note change in appearance and clouring of the root tips
- Fig. 2. — Roots of *Tradescantia* after 2 hours in 0.2% *Poria* extract, kept for several days in water. Evident are the regions without root hairs whose growth had been inhibited. Below these regions root hairs have grown already in water
- Fig. 3. — Prophase in a cell from the root of *Vicia* after 12 hours in 0.8% *Poria* extract. Note despiralization of the chromosomes
- Fig. 4. — Prophase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract and after 24 hours in water. Tape-like bridge-connected agglomerations of vacuolated chromatin
- Fig. 5. — Prophase — as above. Tape-like agglomerations of vacuolated and fragmented chromatin are evident
- Fig. 3, 4 and 5 root tips fixed in acetoalcohol (1:3), smear preparations were stained with acetoorcein. Microphotographs in anoptal contrast. Magnified ca 1500 ×

Plate II

- Fig. 1. — Side view of metaphase in a cell from a control root of *Allium*
- Fig. 2. — Metaphase in smeared cell from a control root of *Allium*
- Fig. 3. — Metaphase in a cell from the root of *Allium* after 24 hours in 0.7% *Poria* extract. Shortening of chromosomes is evident
- Fig. 4. — Metaphase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract. Shortening of chromosomes to vacuolated, granular forms is evident
- Figs. 5 and 6. — Metaphases in cells from the roots of *Allium* after 36 hours in 0.8% *Poria* extract. Diminution of size and vacuolation of chromosomes is evident
- Root tips were fixed in acetoalcohol (1:3). Smear preparations were stained with acetoorcein. Microphotographs of Fig. 4, 5 and 6 in anoptal contrast. Magnified ca 1500 ×

Plate III

Fig. 1. — Anaphase in a cell from a control root of *Allium*.

Fig. 2. — Late anaphase in a cell from the root tip of *Allium* after 24 hours in 0.8% *Poria* extract. Note shortening of chromosomes and "vacuolation" caused probably by despiralization and contraction of chromosomes

Fig. 3. — Early anaphase — as above. Diminution of chromosomes is evident

Fig. 4. — Anaphase in a cell from the root tip of *Allium* after 24 hours in 0.8% *Poria* extract. Diminution of chromosomes as well as constrictions and deformations are evident

Root tips were fixed with acetoalcohol (1:3). Smear preparations were stained with acetoorcein. Microphotographs of Fig. 2, 3 and 4 in anoptal contrast. Magnified ca 1500×

Plate IV

Fig. 1. — Anaphase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract and after 24 hours in water. Note shortening of chromosomes, various types of vacuolation, agglomerations, and deformations

Fig. 2. — Late anaphase in a cell from the root of *Vicia faba* after 48 in 0.8% *Poria* extract and after 24 hours in water. Chromosomes have shortened to spherical forms.

Fig. 3. — Telophase in a cell from the root of *Vicia faba* — as above. Fragmentation of vacuolated chromosomes is visible.

Fig. 4. — Anaphase in a cell from the root of *Vicia faba* — as above. Note diminution and vacuolation of chromosomes

Fig. 5. — Anaphase in a cell from the root of *Allium* after 48 hours in 0.8% *Poria* extract and after 24 hours in water. Diminution, vacuolation, constrictions and fragmentation of chromosomes are evident

Root tips fixed with acetoalcohol (1:3). Smear preparations were stained with acetoorcein. Microphotographs in anoptal contrast. Magnified ca 1500×

Plate I

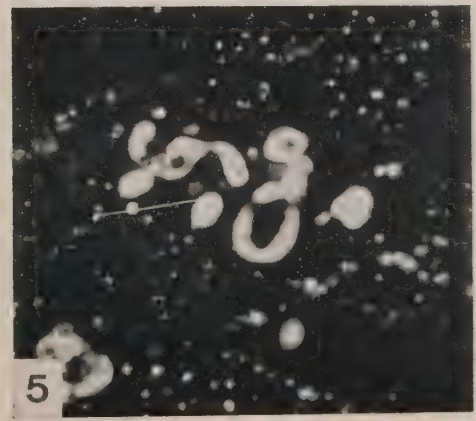
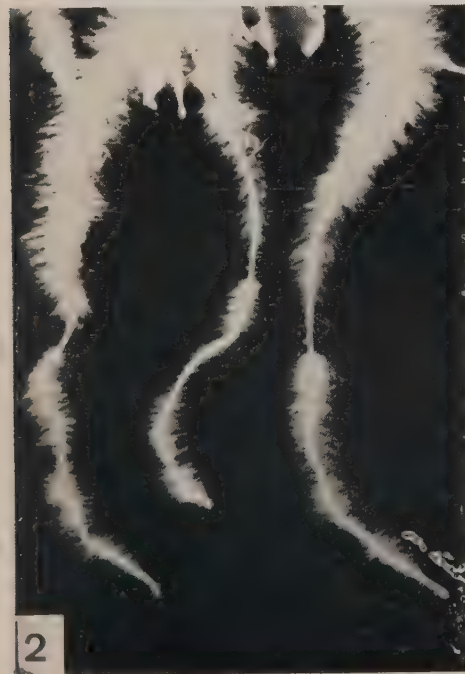


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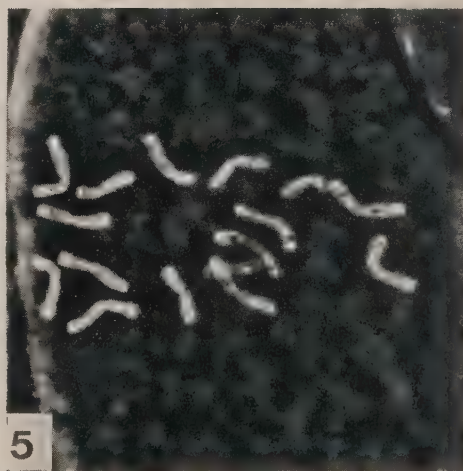
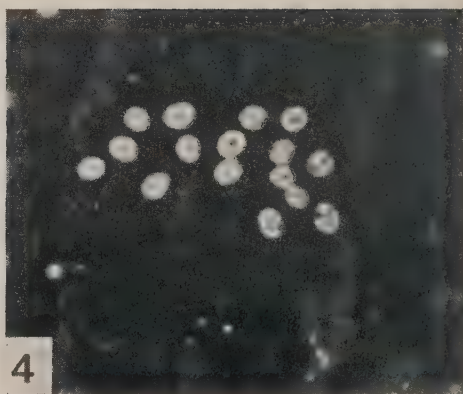
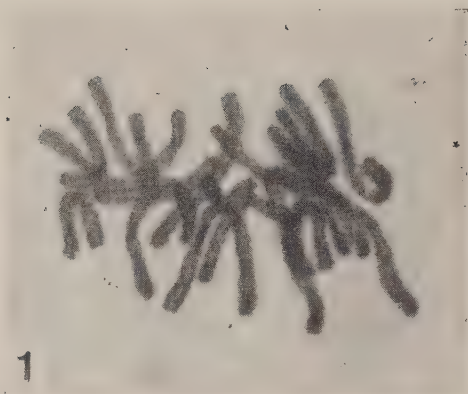


Plate III

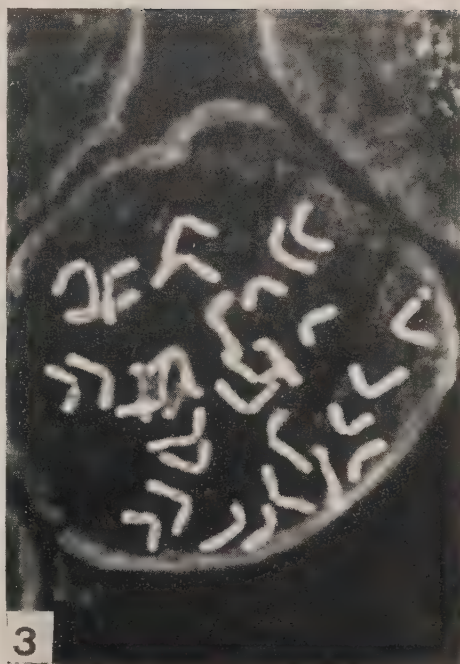
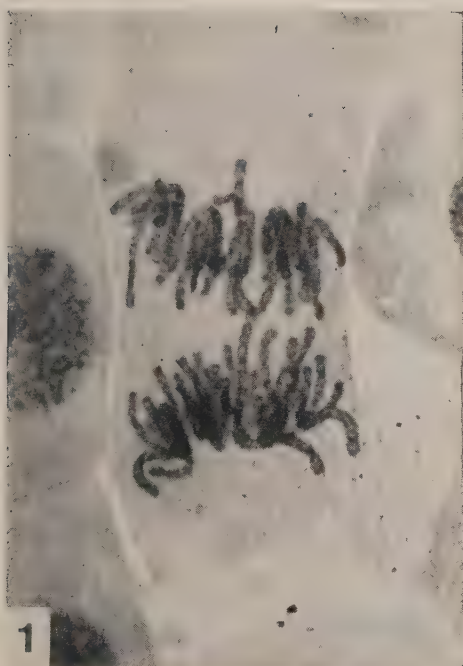
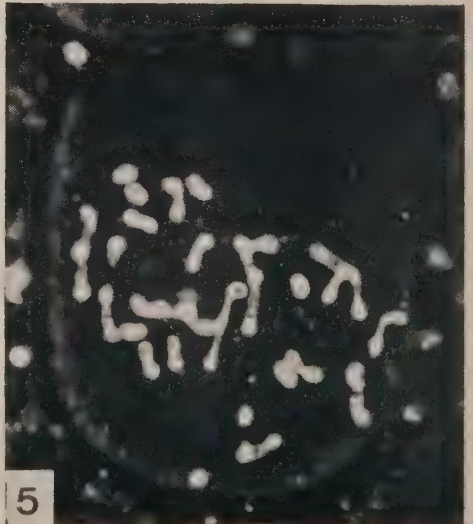
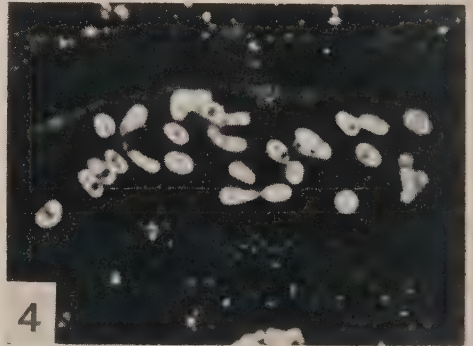
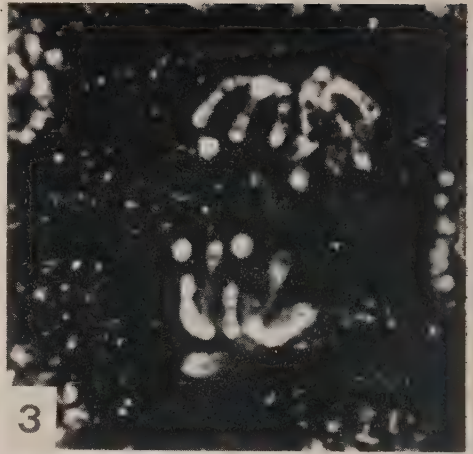
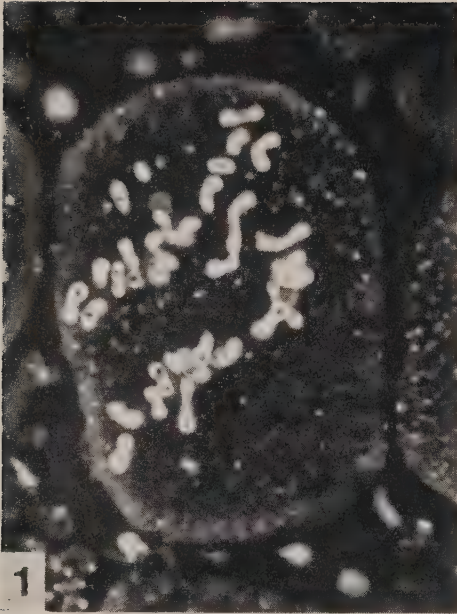


Plate IV



Further additions to chromosome numbers of Polish Angiosperms

M. SKALIŃSKA, M. POTROWICZ, A. SOKOŁOWSKA-KULCZYCKA ET AL.

The present paper gives further additions to the knowledge of the chromosome numbers of Polish Angiosperms, carried out by the research workers of the Institute of Plant Anatomy and Cytology, University of Krakow. The plant material for our studies has been collected only in natural habitats. Like in our previous work (1950, 1957, 1959) the determination of the chromosome numbers was based chiefly on the study of root-tip mitoses.

DICOTYLEDONS

Ranunculaceae

Clematis L. ($x=8$). Studied by A. Sokołowska-Kulczycka.
Clematis alpina (L.) Mill. ($2n=16$).

This species occurs in Poland chiefly in lower mountain habitats in woods and in scrubs. It is particularly frequent in the Tatra Mts. Plants for the present study have been collected by Dr. Z. Radwańska-Paryska in the valley of the lake Morskie Oko. The chromosome number $2n=16$ agrees with the results of previous investigations (Sakai 1935, Meurman and Therman 1939, Gregory 1941, as well as Mattick, in Tischler 1950).

Details of the chromosome morphology of several other species of the genus *Clematis* have been studied by Meurman and Therman; according to the present results, the chromosome set of *C. alpina* seems to be similar to that of *C. viticella* analysed by these authors. It consists of five pairs of large chromosomes with a median or submedian centromere (corresponding to the chromosomes A, B, C, D, E of Meurman and Therman), one pair of large chromosomes with a subterminal centromere (chromosomes F), one pair of large chromosomes with trabants (chromosomes G) and one pair of small chromosomes with a terminal centromere (chromosomes H). In *C. alpina* trabants could be observed only in the chromosomes G (Fig. 1).

Ranunculus L. ($x = 7, 8$).

Ranunculus polyanthemus L. ($2n = 16$). Studied by M. Skalińska.

A species occurring in Poland both in the lowland and in lower mountain habitats, at wood borders, in meadows as well as on dry slopes. The plants for the present study have been collected on sunny hills in Grzybów, near Krakow and in damp meadows near Mokrzyska (N. lat. $50^{\circ} 2'$, E. long. $20^{\circ} 38'$). The number $2n = 16$ agrees with the results of Larter (1932), of Mattick (in Tischler 1950) and of Reese (1953), whereas Felföldy (1947 cited from Tischler 1950) reported the number $2n = 14$.

Ranunculus oreophilus M. B. ($2n = 16$). Studied by A. Sokołowska-Kulczycka.

This is an oreophytic species which in Poland is limited to the Carpathian range where it occurs in mountain meadows and on limestone

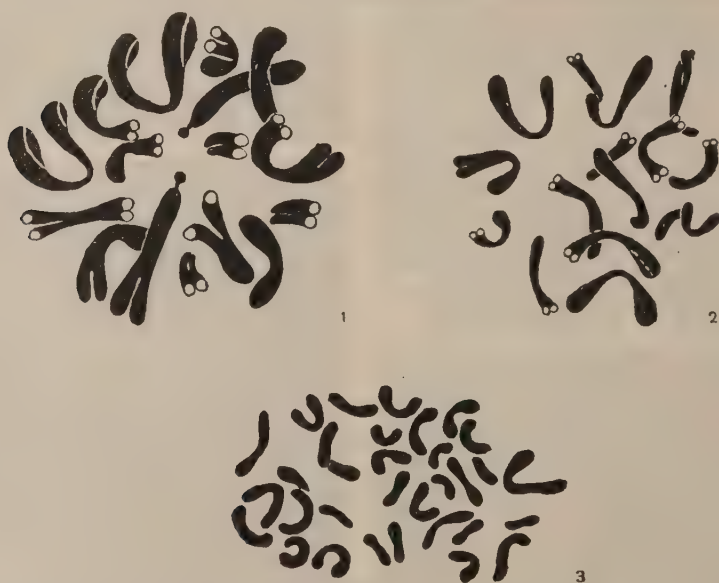


Fig. 1—3. Root-tip metaphases: 1 — *Clematis alpina* ($2n = 16$); 2 — *Ranunculus oreophilus* ($2n = 16$); 3 — *Ranunculus sceleratus* ($2n = 32$) ($\times 3500$)

rocks. The plant material for the present study has been collected in the Tatra Mts by Dr. Z. Radwańska-Paryska. The chromosome number of this species which is related to *R. montanus* L. has not been reported hitherto. It should be added that the specific name of *R. oreophilus* has been given by various authors as a synonyme of several different species (cf. Gregory 1941); none of these species however does correspond with the specimens from the Tatra Mts studied in the course

of the present study. The plants investigated were diploid ($2n = 16$). (Fig. 2).

Ranunculus sceleratus L. ($2n = 32$). Studied by A. Sokołowska-Kulczycka.

This species is common in Poland both in the plain and in lower mountain habitats; it occurs in swamps and on water borders. Plants for the present study originated from two habitats in south-eastern Poland near Lublin, bank of the river Bystrzyca (N. lat. $51^{\circ} 20'$, E. long. $22^{\circ} 35'$) and bank of the canal joining the rivers Wieprz and Krzna (N. lat. $51^{\circ} 45'$, E. long. $22^{\circ} 30'$). The tetraploid chromosome number (Fig. 3) agrees with that reported previously by Langlet (1936), Gregory (1941), Felföldy (1947) and by other authors. The chromosomes of this species are notably smaller than those of other *Ranunculus*-species.

Ranunculus auricomus L. ($2n = 32$). Studied by A. Kleczkowska.

Ranunculus cassubicus L. ($2n = 24, 32, 44, 64$). Studied by J. Kalita.

The huge apomictic complex *Ranunculus auricomus-cassubicus* is split up into a great number of constant biotypes (microspecies) occurring in various parts of its large area of distribution. Some taxonomists give to the various forms the rank of species (W. Koch), while others regard them as subspecies (Marklund) or agamospecies (Gustafsson). In our study we follow Marklund's taxonomical treatment.

The morphological differentiation within this apomictic complex in Poland has been studied by Jasiewicz (1956) who described 11 forms as separate species. Two belong to *R. auricomus* and 9 to *R. cassubicus*. The karyological studies started in the course of the present work deal with a total of 22 populations of *R. auricomus*; 15 could be assigned to *ssp. binatus* (*R. binatus* Kit. em. Jas.) and comprised 7 lowland populations as well as 8 populations from lower mountain habitats; *ssp. sub-tatricus* (*R. subtriticus* Jas.) was represented by 7 populations originating from lower mountain habitats in the Tatra Mts (940 to 1250 m o.s.l.). All plants studied were tetraploid ($2n = 32$) (Fig. 4).

R. cassubicus has been studied on plants belonging to 27 separate populations from southern and central Poland. 13 populations contained forms similar to the *ssp. marginicolus* (*R. marginicolus* Jas.) and 8 could be identified as *ssp. Pawlowskii* (*R. Pawlowskii* Jas.); on the other hand, the remaining six populations could not be assigned with certainty to any of the forms described by Jasiewicz. Contrasting with *R. auricomus* which in Poland seems to be uniform in respect of the chromosome number, *R. cassubicus* shows a remarkable karyological differentiation. The most frequent type occurring here is also the tetraploid; *ssp. Pawlowskii* as well as most undefined populations seem to consist only of tetraploids.



Fig. 4—7. Root-tip metaphases: 4 — *Ranunculus auricomus* ssp. *binatus* ($2n = 32$); 5—7. *Ranunculus cassubicus*, biotypes near to ssp. *marginicolus*; 5 — a triploid biotype ($2n = 24$); 6 — a tetraploid biotype ($2n = 32$), 7 — an aneuploid biotype ($2n = 44$) ($\times 2500$)

On the other hand, in biotypes near to ssp. *marginicolus* several chromosomic types could be found, sometimes in the same population: besides the tetraploids also plants with the aneuploid number $2n = 44$ have been found repeatedly (Figs 6, 7). It should be added that this type has been detected also in some of the populations which could not be assigned to any of the forms described by Jasiewicz. Other cytotypes occurring among biotypes near to ssp. *marginicolus* seem to represent very rare exceptions: in one habitat a single octoploid ($2n = 64$) as well as a triploid plant ($2n = 24$) growing in close neighbourhood of the tetraploid and the aneuploid were found (Fig. 5).

The occurrence of the aneuploid type is the more interesting, as in studies of previous authors dealing with the cytology of the *Ranunculus auricomus-cassubicus* complex only strictly euploid numbers have been reported. Rutishauser (1954) has found exclusively tetraploids in the Swiss populations. In addition, occasionally pentaploids with 40 somatic chromosomes and hexaploids with 48 chromosomes have been found by Häfliger (1943) in Switzerland and by Rousi (1956) in Finland.

Another unexpected type, the origin of which is difficult to elucidate is represented by the single triploid plant. On the other hand, the octoploid which has been found also only once, might have arisen spontaneously in the way of chromosome doubling in some vegetative parts of a plant; this higher degree of polyploidy could have been fixed subsequently by apomixis.

In view of the results obtained, the study is being continued.

Rosaceae

Comarum L. ($x = 7$). Studied by A. Sokołowska-Kulczycka.

Comarum palustre L. ($2n = 42$).

This species which has a wide general area of distribution, is rather frequent in Poland in the plain but is relatively rare in lower mountain habitats. It grows on moist ground in peat-moors and at borders of stagnant waters. The plant material for the present study has been collected in the following six habitats: Jastrzębia Góra, Baltic coast (N. lat. $54^{\circ}45'$, E. long. $18^{\circ}20'$), coll. Dr. E. Pogan; swampy ground at the border of the lake Wygoda, south of Wejherowo, Pomeranian Lake district (N. lat. $54^{\circ}32'$, E. long. $18^{\circ}15'$) coll. Prof. M. Skalińska; border of the

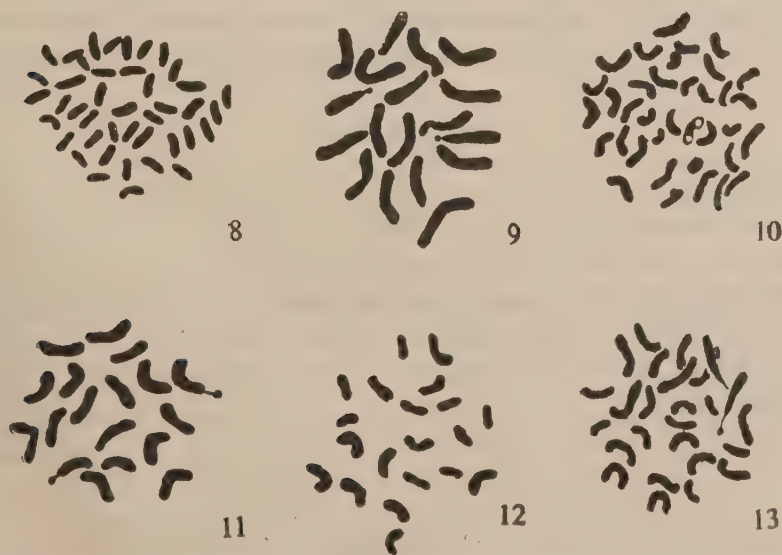


Fig. 8—13. Root-tip metaphases: 8 — *Comarum palustre* ($2n = 42$) ($\times 3500$); 9 — *Cakile maritima* ($2n = 18$); 10 — *Spergularia salina* ($2n = 36$) ($\times 3700$); 11 — *Salicornia herbacea* ($2n = 18$); 12 — *Hottonia palustris* ($2n = 20$); 13 — *Samolus Valerandi* ($2n = 26$) ($\times 3600$)

lake Podkówka, near Olsztyn, Masovian lake district (N. lat. $53^{\circ}45'$, E. long. $20^{\circ}30'$) coll. Dr. E. Pogan; borders of the lakes Czarne and Białe in the Lublin lake district (N. lat. $51^{\circ}20'$, E. long. $23^{\circ}30'$); swampy ground in the vicinity of Wieluń (N. lat. $51^{\circ}15'$, E. long. $18^{\circ}45'$).

In all the above habitats exclusively plants with the hexaploid chromosome number ($2n = 42$) have been found (Fig. 8). It should be emphasized that previous investigations in this species revealed the occurrence of a polyploid differentiation: the lowest number corresponding with the tetraploid type ($2n = 28$) has been reported from Germany (Wulff 1937), from Scandinavia (Ehrenberg 1945) as well as from Iceland (Löve a. Löve 1956); the occurrence of hexaploids has been reported from SW-Greenland by Jørgensen, Sørensen and Westergaard (1958) and by Sokolovskaja and Strelkova (1941) from the district of Leningrad; the latter authors have found in addition biotypes with a still higher number, viz. $2n = 64$, in the arctic (isle of Kolguyev).

Papilionaceae

Lathyrus L. ($x = 7$). Studied by M. Piotrowicz.

Lathyrus maritimus (L.) Big. ($2n = 14$).

A common species occurring on sand dunes of the Baltic coast. The plants studied originated from two habitats on the Hel peninsula (Jastarnia and Kuźnica). Their chromosome number $2n = 14$ agrees with that reported by Senn (1938) and by other authors, e.g. by Wulff (1950).

Cruciferae

Cakile Mill. ($x = 9$). Studied by M. Piotrowicz.

Cakile maritima Scop. ($2n = 18$).

This species occurs in Poland only at the Baltic coast on sandy soil. The specimens for the present study originated from the Hel peninsula (Jastarnia, Kuźnica). The number $2n = 18$ (Fig. 9) agrees with that previously reported by Jaretsky (1932), by Manton (1932) and other authors.

Caryophyllaceae

Spergularia Presl. ($x = 9$). Studied by M. Piotrowicz.

Spergularia salina Presl. ($2n = 36$).

In Poland this halophytic species is a rather common component of the flora of salines in various parts of the country. The specimens studied have been collected in salt marshes along the Baltic coast (Wielka Wieś

and Swarzewo). The tetraploid number ($2n=36$) (Fig. 10) is in accordance with the results of previous authors quoted by Tischler (1950).

Chenopodiaceae

Salicornia L. ($x=9$). Studied by H. Jaworska*.

Salicornia herbacea L. (*S. europaea* L.). ($2n=18$).

This halophytic species is the only representative of the genus in the flora of Poland. It occurs in salt marshes along the Baltic coast as well as in inland salines. The plants for the present study originated from the inland salines in central Poland (Ciechocinek and Inowrocław, south of Toruń); they have been collected on banks of a ditch and in meadows overflowed by water from salt-works; in these habitats they occur together with some other halophytic species.

In all plants from the above habitats the number $2n=18$ could be established. One pair of chromosomes bears trabants (Fig. 11).

According to results of previous studies *S. herbacea* shows some degree of intraspecific karyological differentiation. Tarnavski (1938) reported likewise $2n=18$ for specimens from Rumania, whereas Wulff (1937a) found in plants from Schleswig-Holstein a higher number ($2n=38$) considered by him as hypertetraploid. On the other hand König (1939, cited from Tischler 1950) has found two different types, the diploid as well as the tetraploid one ($2n=36$). In view of this differentiation, further studies of specimens from the salines along the Baltic coast should supplement the present results.

Umbelliferae

Hydrocotyle L. ($x=12$). Studied by A. Sokołowska-Kulczycka.

Hydrocotyle vulgaris L. ($2n=c. 96$).

This species which is rare in Poland occurs in peat-bogs and swamps chiefly in the western part of the country, while more eastwards it is found only in dispersed habitats. The plants for the present study originated from the south-eastern part of Poland in the Lublin lake district (N. lat. $51^{\circ}20'$, E. long. $23^{\circ}30'$), a large area with many lakes and an abundant watering of the soil.

The chromosome number of this species was estimated previously by Wanscher (1932, 1933, cited from Tischler 1950) as $2n=c. 96$, and the same result has been obtained by Håkansson (1953) and by

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Löve and Löve (1956). In view of this high number of chromosomes and their approximately uniform size, it proved difficult also in the course of the present study to obtain more exact results; the chromosome number of the Polish plants however seems to correspond with that found by Wanscher and by Håkansson.

Primulaceae

Hottonia L. ($x=10$). Studied by A. Sokołowska-Kulczycka.

Hottonia palustris L. ($2n=20$).

This species is widely distributed in Poland in stagnant waters. The plants for the present study originated from two habitats in the north of Poland: in peat moors Błota Bielawskie near Puck (coll. Dr. E. Pogan) and Błota Karwińskie (coll. J. Małecka).

The chromosome number $2n=20$ (Fig. 12) agrees with that previously reported by Wulff (1938) and by Ehrenberg (1945).

Glaux L. ($x=15$). Studied by M. Skalińska.

Glaux maritima L. ($2n=30$).

This halophytic species is very common in Poland in salt marshes along the Baltic coast; it occurs also in inland salines. The plants for the present study have been collected in three habitats: in salt marshes at the Baltic coast in Wielka Wieś near the bay of Puck; on the Hel peninsula near Jastarnia; and near Swarzewo, somewhat farther from the coast.

The chromosome number $2n=30$ agrees with that previously reported by Wulff (1937) for plants from Schleswig-Holstein and by Tarnavski (1938) from Rumania, as well as by Löve and Löve (1956) from Iceland.

Samolus L. ($x=13$). Studied by M. Skalińska.

Samolus Valerandi L. ($2n=26$).

In the flora of Poland *Samolus Valerandi* represents a very rare halophytic species, the occurrence of which is limited only to some isolated habitats at the Baltic coast in salt marshes; it appears also, as a rarity, in some inland salines. The plants for the present study have been collected in salt marshes near the Baltic coast in the vicinity of Wielka Wieś at the Bay of Puck, where they grow abundantly.

The chromosome number of this species has been studied first by Wulff (1937) who found $2n=c.24$. The same number has been reported recently by Larsen (1960) for representatives of this species from the Canary Islands. Some other authors however have determined the chromosome number of this species as $2n=26$ (Tarnavski 1948,

Rodrigues 1953). The same number has been found also for plants from Poland studied in the course of the present work. The chromosomes show distinct size differences. One pair of chromosomes bears trabants (Fig. 13).

Rubiaceae

Galium L. ($x=11, 12$). Studied by M. Piotrowicz.

Galium valdepiosum H. Braun. ($2n=22$).

This species occurs in central Europe in dispersed habitats from north-western Lower Austria to Moravia and Bohemia; and along the Danube through Upper Austria to eastern Bavaria (Ehrendorfer 1956). The studies of this author who regards it as an endemical subspecies (*ssp. valdepiosum*) revealed some degree of karyological differentiation in the above area: in Moravia and north-western Austria chiefly diploids were found by him, whereas in Bohemia and eastern Bavaria only tetraploid types seem to occur.

In Poland this species has been found on the Plateau of Little Poland where it occurs on sunny calcareous slopes in grassland among thermophilous plants. The material for the present study has been collected on hills near Miechów; Zarogów near Pojałowice (coll. B. Pawłowski) as well as in the steppe reservation Sterczów-Ścianka and on the slope of hill Grodzisko (coll. I. Kucowa). All plants investigated proved to be diploid ($2n=22$). This result is interesting in view of the details concerning the distribution of diploid biotypes of the *ssp. valdepiosum* reported by Ehrendorfer (l.c.).

Galium boreale L. ($2n=44, 55, 66$).

In a previous paper (Skalińska, Czapik, Piotrowicz 1959) the hexaploid number ($2n=66$) has been reported by the author for plants from two habitats in Poland. Further studies have revealed some degree of karyological differentiation within this species in Poland. In addition to that number, two other numbers, viz. $2n=44$ (Fig. 14) and $2n=55$ (Fig. 15) have been found.

Biotypes with $2n=44$ were found in Izabelin near Warsaw; they grew in wood clearings, whereas in rather shady habitats in the same wood biotypes with $2n=66$ have been found. The pentaploid type ($2n=55$) has been detected in Iznota, near Olsztyn, NE Poland (coll. K. Bijok) in a mixed population together with hexaploid plants.

The number $2n=44$ agrees with that previously reported from other areas of Europe by Fagerlind (1934, 1937), Poucques (1949), Böcher and Larsen (1950) as well as by Löve (1954) and by

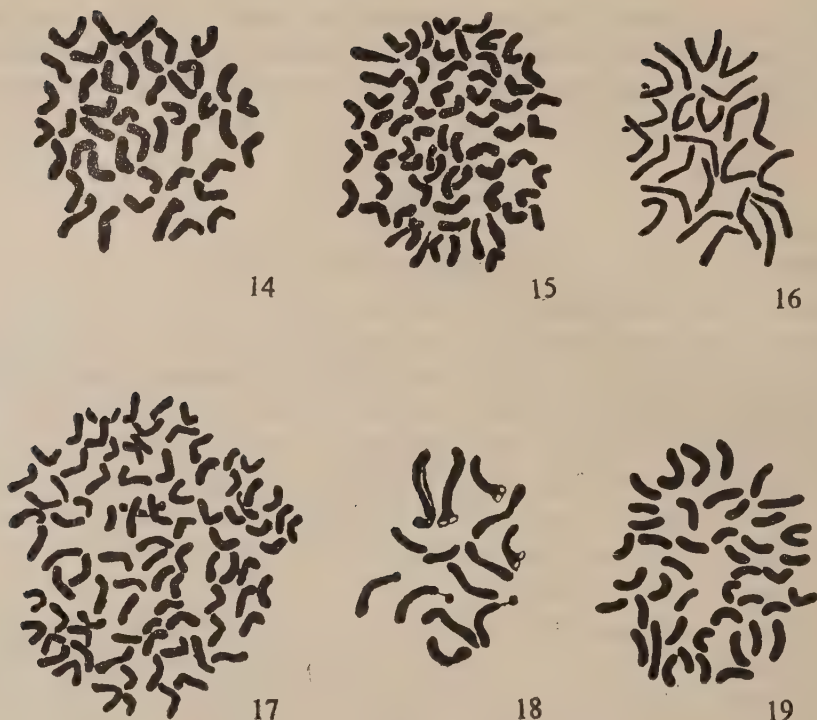


Fig. 14—19. Root-tip metaphases: 14, 15 — *Galium boreale* ($2n = 44$ and 55); 16, 17 — *Galium palustre* ($2n = 24$ and 88) ($\times 3700$); 18 — *Lobelia Dortmanna* ($2n = 14$); 19 — *Centaurea montana* ssp. *mollis* ($2n = 44$) ($\times 3000$)

Löve and Löve 1954, 1956). On the other hand, the pentaploid number is given here for the first time.

It should be added that the occurrence in Europe of the hexaploid *G. boreale* has been queried by some authors. Therefore it seems important to note that this number ($2n = 66$) has been found again in the course of the present study in three new well separated habitats: central Poland near Warsaw, NE Poland near Olsztyn and N Poland at the Baltic coast near Karwia (N. lat. $54^{\circ}48'$, E. long. $18^{\circ}7'$). These data supplement our previous results (1959) based of the study of two habitats: N. Poland and S. Poland in the vicinity of Krakow. Thus, the hexaploid biotypes seem to be rather common in Poland.

Galium palustre L. ($2n = 24, 88$).

This species is common in all parts of Poland in damp meadows, in marshes and banks of ditches. In the course of the present study plants from seven habitats have been investigated. The results have shown the occurrence of two different chromosomic types. The first is represented by plants with the number $2n = 24$ (Fig. 16); they originated from

a single habitat near Jastarnia on the Hel peninsula. This chromosome number has been reported previously by Fagerlind (1934, 1937), Hagerup (1941), and by Hancock (1942, cited from Darlington and Wylie, 1955). The second chromosomic type was $2n=88$ (Fig. 17). Plants belonging to the latter type have been collected in six habitats: near Karwia at the Baltic coast (three separate habitats), near Jastarnia on the Hel peninsula, Izabelin near Warsaw and Sosnowica near Lublin.

Previous studies in *G. palustre* led to establish a rather complex intraspecific karyological differentiation. The following numbers have been reported in addition to 24: $2n=48$ (Fagerlind 1937, Hancock 1942), $2n=66$ ($n=33$, Homeyer 1936); for *var. elongatum* G. Beck $2n=95?$ (Fagerlind 1934, 1937) $2n=96$ (Hancock 1942); and for *var. aparinoides* $2n=c.100$ (Fagerlind 1937). These cytotypes however have not been found in the course of the present study. On the other hand, the type with 88 somatic chromosomes has been detected for the first time in plants from natural habitats in Poland.

The available data concerning the karyological differentiation of *G. palustre* suggest the occurrence of two basic numbers, $x=11$ and 12, within this species. In view of the present results a more detailed cytological study of this species in Poland has been undertaken.

Plantaginaceae

Plantago L. ($x=6$) Studied by D. Czapska.

Plantago maritima L. ($2n=12, 18$).

In Poland this species is confined only to salt marshes along the Baltic coast. The plant material for the present study has been collected in various natural habitats extending from the isles of Uznam and Wolin in the western parts of Pomerania along the Baltic coast — to the Bay of Puck.

The chromosome number of *P. maritima* has been studied hitherto on plants originating from various parts of its area of distribution. Previous counts were done by McCullagh on plants from NW Greenland (in Gregor, 1939), by Löve and Löve (1956) from Iceland, by Jørgensen, Sørensen and Westergaard (1958) for SW Greenland, by Wulff (1937) from Sylt and by Tarnavski (1938) from the Black Sea coast in Rumania. All these authors have reported exclusively the diploid chromosome number $2n=12$. The majority of the plants studied in the course of the present work proved to be, likewise, diploid. In one habitat however, on the Hel peninsula in the vicinity of Jastarnia in a salt marsh at the border of the Bay of Puck, triploid plants

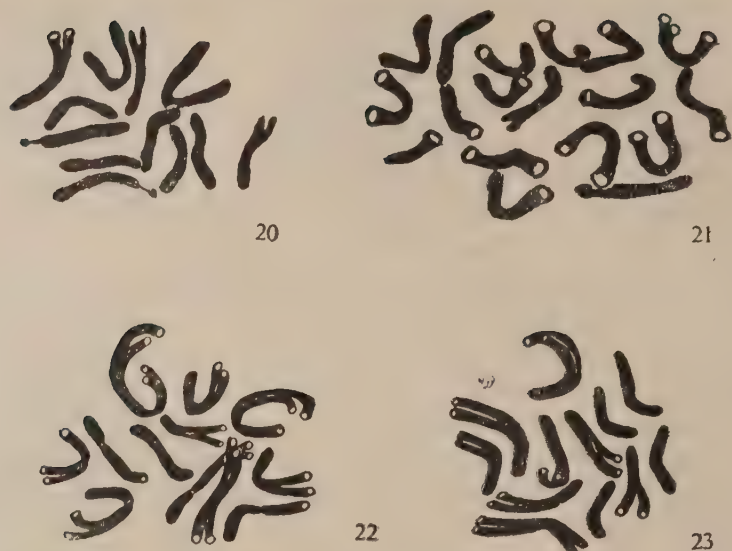


Fig. 20—23. Root-tip metaphases: 20,21 — *Plantago maritima* ($2n=12$ and 18) ($\times 2800$); 22 — *Tragopogon orientalis* ($2n = 12$); 23 — *Tragopogon pratensis* ($2n = 12$) ($\times 2880$)

with $2n=18$ could be found in two successive years (Fig. 20, 21). The occurrence of this chromosomic type is interesting in view of the ability of a vegetative propagation which may contribute to establish these rare triploids in their natural habitats. The study is being continued.

Lobeliaceae

Lobelia L. ($x=7$). Studied by M. Skalińska.

Lobelia Dortmanna L. ($2n=14$).

In Poland the occurrence of this amphiatlantic species is limited to the district of postglacial lakes in western Pomerania. It grows there abundantly in shallow oligotrophic waters. Plants for the present study originated from their native habitat in the lake Wygoda south of Wejherowo (N. lat. $54^{\circ}32'$, E. long. $18^{\circ}15'$); they were collected by Dr. K. Mianowska and M. Skalińska.

The chromosome number $2n=14$ agrees with the recent reports of Reese (1952) and of Bowden (1958). Two chromosomes have distinct trabants (Fig. 18). According to Bowden, species of the genus *Lobelia* show a polyploid differentiation. In his studies dealing with the phylogeny of the genus this author expresses the opinion that *L. Dortmanna* belongs to the oldest species within this genus.

Compositae

Centaurea L. Studied by R. Czapik.

Centaurea montana L. ssp. *mollis* (W. et K.) Gugler ($2n=44$).

C. montana ssp. *mollis* occurs in lower as well as higher mountain habitats in the Carpathians. It grows on calcareous soil only, in scrubs on grassy slopes and at banks of streams. For the present study plants from three habitats have been collected by Dr. Z. Radwańska-Paryska on the slopes of Podskalki (c. 1200 m.o.s.l.) in the Bélan Tatra (ČSR). Their somatic number $2n=44$ (Fig. 19) agrees with the results of Baksay (1957) dealing with specimens from Hungary. This species belongs to the group of *Centaurea* species with the basic number $x=11$.

Tragopogon L. ($x=6$). Studied by H. Jaworska.

Tragopogon maior Jacq. (*T. dubius* Scop.) ($2n=12$).

A species occurring in southern and central Poland in rather dry habitats. The plant material for the present study has been collected by M. Skalińska near Warsaw on a dry grassy slope on the right bank of river Vistula. The chromosome number $2n=12$ agrees with that previously reported by Pólya (1950) for plants from Hungary, as well as by Mulligan (1957) for specimens from Canada.

Tragopogon pratensis L. ($2n=12$).

This species is widespread in Poland chiefly in the lowland in meadows and along roadsides; it occurs also in lower mountain habitats. Plants for the present study originated from two habitats in the vicinity of Warsaw: dry meadows on hills in Bielany, on the left bank of river Vistula, and a roadside along the right bank of Vistula.

The chromosome number $2n=12$ (Fig. 23) is in accordance with the results of Winge (1938) and of Wulff (1950), as well as those of Mulligan (1957) who studied this species in Canada.

Tragopogon orientalis L. ($2n=12$).

T. orientalis, regarded by some taxonomists as a subspecies of *T. pratensis*, occurs in Poland in the lowland in meadows and on roadsides while in the Carpathians it ascends to the zone of *Pinus montana*.

The plants for this study originated from two habitats: Starogród near Chełmno on Vistula (N. lat. $53^{\circ}20'$, E. long. $18^{\circ}30'$), roadside along fields, and Piwnice near Toruń.

The number $2n=12$ (Fig. 22) found in the plants studied is the same as that recently reported by Mulligan (1957).

Chrysanthemum L. ($x=9$). Studied by E. Gacek.

Chrysanthemum leucanthemum L. ($2n=18, 36$, c. 54).

According to the results obtained by Böcher and Larsen (1957), by Baksay (1957) and by Favarger (1959), this species shows a well

marked karyological differentiation in connection with ecology and geographic distribution. The present study deals with 30 populations originating from various parts of Poland. The most frequent type is represented by tetraploids (24 populations) which are widespread over all Poland, both in the plain and in lower mountain habitats. On the other hand, diploid populations are notably rarer. They are represented by two different ecological types. The first has been found, in accordance with the report of Böcher and Larsen (1957), in the vicinity of Puszczykowo; they occur there very locally on a sunny slope of Osowa Góra. In addition, in the same region a second habitat has been found in Luboń near Poznań. This type seems to correspond with the *ssp. praecox* of Horvatič. Another diploid type quite different in respect of ecology has been detected in the range of Babia Góra (Western Carpathians) in two habitats at the altitude of c. 1500 m o.s.l. on stony slopes bearing only a very scanty vegetation. It represents the *ssp. alpicola*, recently studied by Favarger (1959) in mountain habitats in Switzerland. In addition, a third chromosomic type has been found hitherto only in four habitats in the Western Tatra at altitudes ranging from c. 950 to 1250 m o.s.l. The plants grew in mountain meadows and in pastures. Their chromosome number is c. 54, thus they correspond presumably with the hexaploid types reported by Baksay for the Tatra Mts of Slovakia and by Favarger from the Swiss Alps and the Jura Mts.

Further studies are in progress.

MONOCOTYLEDONS

Alismataceae

Alisma L. ($x=7$). Studied by E. Banach-Pogan.

Alisma plantago-aquatica L. ($2n=14$).

Alisma lanceolatum With. ($2n=26$).

Of the three representatives of the genus *Alisma* in the flora of Poland, viz. *A. plantago-aquatica*, *A. lanceolatum* and *A. gramineum* Gmel. the two former have been investigated in the course of the present study. *A. plantago-aquatica* is very common and occurs over all Poland; by contrast, *A. lanceolatum* is rather rare in our flora.

Plant specimens of *A. plantago-aquatica* were collected in 61 natural habitats distributed over the whole area from the Baltic coast to the foreland of the Tatra Mts. In 10 of these habitats *A. lanceolatum* occurred together with *A. plantago-aquatica*; the two species grew there in close neighbourhood, not unfrequently in the same tufts.

The genus *Alisma* shows some degree of karyological differentiation. Previous studies led to establish the following chromosome numbers: *Alisma plantago-aquatica*: $2n = 10$ (Wulff 1939); $2n = 12$ (Liehr 1916, Löve and Löve 1942, Palmgren 1943, Blomstrand, in Löve and Löve 1944); $2n = 14$ (Heppel, in Maude 1939, Blomstrand, in Löve and Löve 1944, Hagerup 1944, Erlandsson 1946, Löve and Löve 1948, Tschermak-Woess 1948, Pólya 1949, Wulff 1950, Holzer 1952, Löve 1954, Hendricks 1957); $2n = 16$ (Wulff 1950); $2n = 28$ (Wulff 1950).

Alisma lanceolatum: $2n = 26$ (Löve and Löve 1944, Tschermak-Woess 1948); $2n = 28$ (Hagerup 1944, Erlandsson 1946, Löve and Löve 1948).

In the course of the present investigation only one chromosome number was invariably found for each species in all specimens studied, namely $2n = 14$ for *A. plantago-aquatica* and $2n = 26$ for *A. lanceolatum*. Further studies, now in progress, will presumably throw light upon the type of karyological differentiation within this genus.

Butomaceae

Butomus L. ($x = 13$). Studied by M. Skalińska.

Butomus umbellatus L. ($2n = 26$).

This species which is a member of the swamp-flora, is rather common in the lowland of Poland where it grows particularly in shallow water near the borders of lakes and ponds. Plants for the present study originated from two habitats: in southern Poland — Jadowniki Mokre (N. lat. $50^{\circ}15'$, E. long. $20^{\circ}40'$) in a lake formed in the ancient bed of river Vistula; in northern Poland — Fordon on Vistula (N. lat. $53^{\circ}15'$, E. long. $18^{\circ}15'$) in swampy ground at the edge of a pond.

The chromosome number $2n = 26$ agrees with one of the two numbers found by Lohammar (in Löve and Löve 1948). The triploid number $2n = 39$ has been also reported by this author. Within the somatic plate distinct size differences of the chromosomes are observable (Fig. 24). Three pairs of large heterobrachial chromosomes (5.5 to 7μ), a pair of somewhat smaller chromosomes (c. 5μ), one pair of small (c. 2.5μ) and eight pairs of very small chromosomes (1 to 1.6μ) could be discerned.

Hydrocharitaceae

Hydrocharis L. ($x = 14$). Studied by A. Sokołowska-Kulczycka.

Hydrocharis morsus-ranae L. ($2n = 28$).

This is a common species occurring in stagnant waters over all the

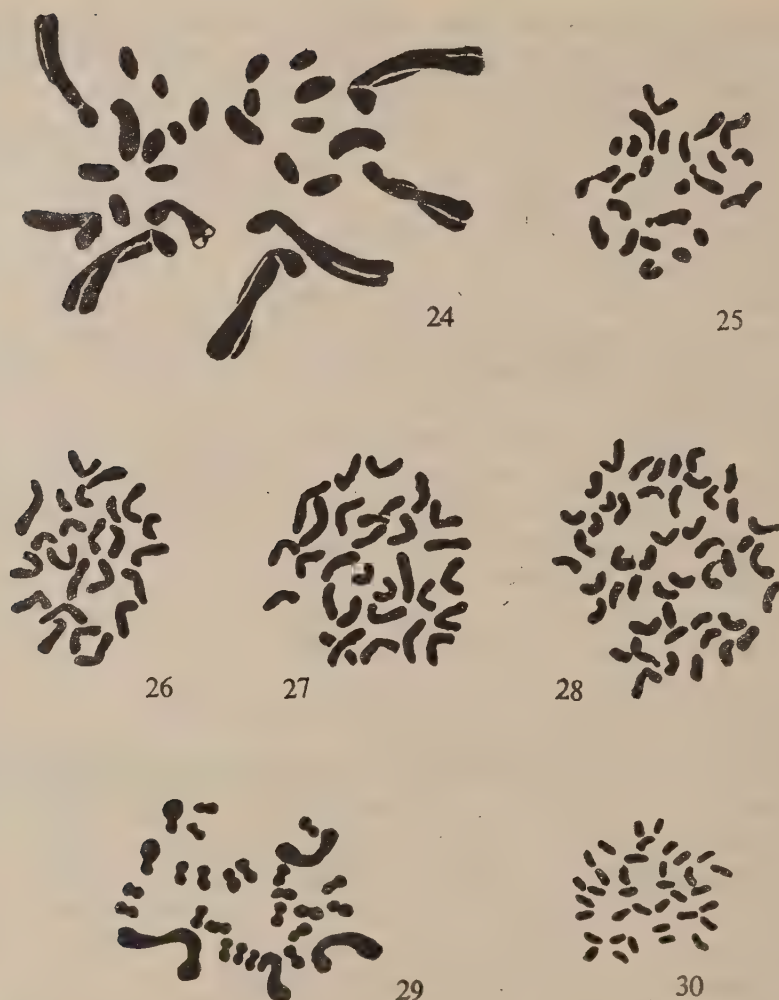


Fig. 24—30. Root-tip metaphases: 24 — *Butomus umbellatus* ($2n = 26$) ($\times 3000$); 25 — *Triglochin palustre* ($2n = 24$) ($\times 3700$); 26, 27, 28 — *Triglochin maritimum* ($2n = 24, 30, 48$) ($\times 3700$); 29 — *Hydrocharis morsus-ranae* ($2n = 28$); 30 — *Acorus calamus* ($2n = 36$) ($\times 3500$)

Polish plain. The material for the present study has been collected by Z. Trela in lakes near Jadowniki Mokre (southern Poland).

The chromosome number $2n = 28$ agrees with the previous results of Tuschniakova (1929), of Hoare (in Maude 1939) and of Ehrenberg (1945). It should be added that within the somatic plates the chromosomes are distinctly differentiated in respect of their length; the following types may be discerned: two pairs of larger chromosomes

(c. 4μ and 3μ resp.), two pairs of smaller chromosomes (c. 1.7μ) and eight pairs of very small chromosomes attaining hardly the length of 0.8μ (Fig. 29).

Juncaginaceae

Triglochin L. ($x=6$). Studied by M. Piotrowicz.

Triglochin palustre ($2n=24$).

This species is rather common in Poland and occurs in damp meadows and at borders of stagnant waters. The material for the present study originated from one habitat in the vicinity of Kraków and from 5 habitats in northern Poland at the Baltic coast; Karwia (two habitats), Swarzewo, as well as Jastarnia and Jurata on the Hel peninsula.

In all specimens studied invariably the chromosome number $2n=24$ (Fig. 25) was found. The same number has been reported previously by Wulff (1939), Löve and Löve (1944, 1956) as well as by Jørgensen, Sørensen and Westergaard (1958). It should be added that in the metaphase plates a pair of chromosomes with distinct secondary constrictions has been observed; it may be easily mistaken for two pairs.

Triglochin maritimum L. ($2n=24, 30, 48$).

A species occurring frequently in damp meadows and salt marshes along the Baltic coast and notably rarer in some inland salines. Plants for the present study have been collected in 8 habitats situated near the Baltic coast. In this material three different chromosome numbers have been found, viz. $2n=24, 30$, and 48 . The number 24 (Fig. 26) has been found in plants from a single habitat near Kolobrzeg at the Baltic coast; the same number has been reported by Tarnavski (1948) for plants from Transsylvania. The chromosome number $2n=48$ (Fig. 28) seems to be the most common at the Baltic coast; it has been found in specimens originating from Wielka Wieś (coll. M. Skalińska), as well as from Karwia, and from Jastarnia and Jurata on the Hel Peninsula. This chromosomic type seems to be widespread also in other parts of Europe; it was reported by Winge (1925), Wulff (1939), Tarnavski (1948), as well as by Löve and Löve (1944, 1956). On the other hand, the number 30 (Fig. 27) found in plants originating from two further habitats on the Hel peninsula (Jastarnia and Jurata) as well as from Swarzewo, is given here for the first time. It represents evidently the pentaploid type. On the whole, *T. maritimum* is a species which shows a high degree of karyological differentiation; in addition to the chromosome numbers quoted above, also the following numbers, not detected hitherto in the habitats from Poland, have been reported previously by

Tarnavski (1938, 1948): $2n=12$ from Rumania and $2n=36$ from Portugal. It should be added that in Japan and in North America notably higher degrees of polyploidy have been attained by this species (cf. Löve and Löve 1958a, b).

Araceae

Acorus L. ($x=12$). Studied A. Sokołowska-Kulczycka.

Acorus calamus L. ($2n=36$).

This species is widespread over all Poland growing in swampy ground at banks of stagnant waters. The material for the present study has been collected in 20 habitats from various parts of Poland (map of habitats,

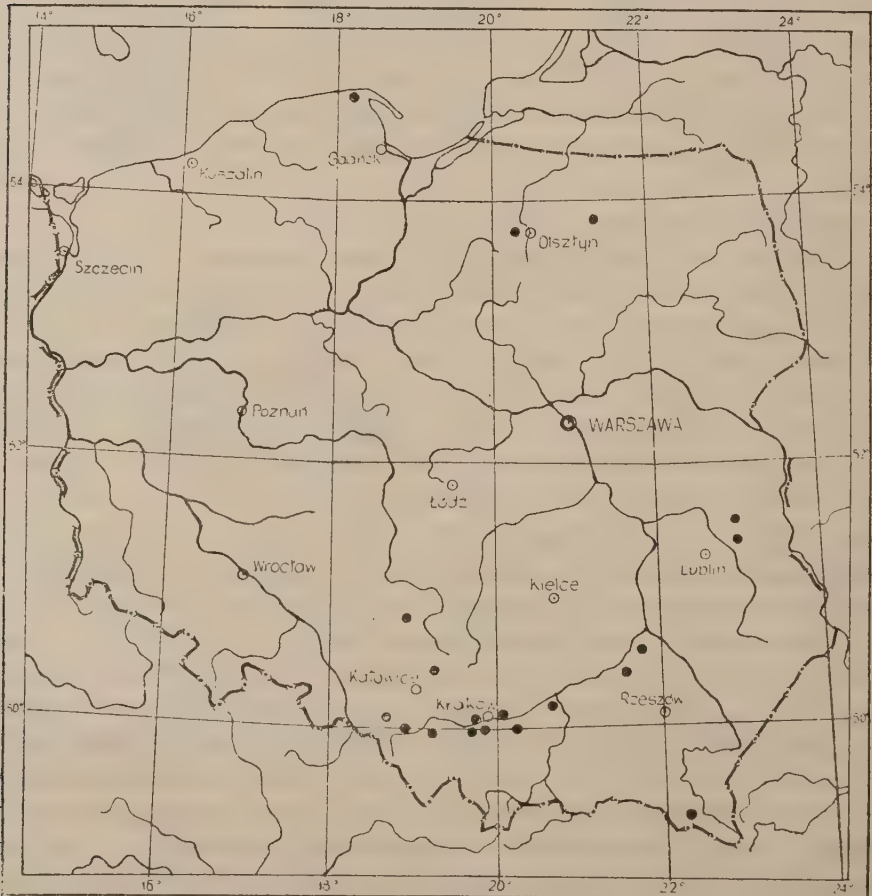


Fig. 31. Map of habitats of *Acorus calamus*

Fig. 31). All plants studied had the triploid chromosome number, $2n = 36$ (Fig. 30).

The studies of Wulff (1954) concerning the chromosome number and the geographic distribution of various chromosomic types led to establish the occurrence in well separated areas of the diploid, the triploid and the tetraploid types. According to this author, the diploid form ($2n = 24$) occurs in North America, whereas in central Europe hitherto only the sterile triploid form has been found. The same type occurs in India. On the other hand, in Asia from western Siberia to Japan *Acorus calamus* is represented by the tetraploid form ($2n = 48$), var. *spurius* (Schott) Engl.

In the course of the present study the possibility of detecting in the south-eastern part of the country a different chromosomic type has been taken into consideration in view of the occurrence of var. *angustifolius* in Galicia (Zapałowicz 1906). According to Wein (cited from Wulff 1954), this variety may possibly correspond with the tetraploid var. *spurius*, native in Asia. However, all biotypes originating from the southern and south-eastern parts of Poland within her present boundaries proved to be triploid in agreement with the results of Wulff.

Liliaceae

Ornithogalum L. ($x = 9$). Studied by R. Czapiak.

Ornithogalum Gussonei Ten. (*O. tenuifolium* Guss.) ($2n = 18$).

According to Szafer, Kulczyński and Pawłowski (1953) natural habitats of this species in Poland are found only in the south: in Silesia, in the district of Miechów and along the lower course of the river Nida. The plants for karyological studies originated from the latter habitat; they have been collected in the steppe reservation "Skorocice" near Busko (N. lat. $50^{\circ}30'$, E. long. $20^{\circ}40'$). The plant associations of this reservation have been studied recently by Medwecka-Kornaś (1949).

All plants studied proved to be diploid ($2n = 18$) (Fig. 32). The same chromosome number has been found in plants from Hungary by Pólya (1949).

Ornithogalum umbellatum L. ($2n = 27$).

This species grows chiefly as a weed in corn-fields and in gardens in southern Poland. According to Kornaś (1950) it may be regarded as a species characteristic of the corn-field association *Arnosereto-Scleranthetum*.

The chromosome number has been established for plants collected in two habitats in the vicinity of Krakow: Batowice and Brzozkwinia



Fig. 32—34. Root-tip metaphases: 32 — *Ornithogalum Gussonei* ($2n = 18$); 33 — *Ornithogalum umbellatum* ($2n = 27$) ($\times 2260$); 34 — *Listera cordata* ($2n = 38$) ($\times 3000$)

(coll. M. Ochlewska) as well as near Dąbrowa Tarnowska (N. lat. $50^{\circ}10'$, E. long. 21°) (coll. D. Tumidajowicz). All these specimens were triploid ($2n = 27$) (Fig. 33). Within this species a polyploid differentiation has been already reported; the following numbers are known: $2n = 18, 27, 36, 45, 54, 72$ (Sprumont 1928, Matsuura and Sutô 1935, cited from Sutô 1936, Nakajima 1936, Pólya 1950, Holzer 1952, Neves 1952, 1956a, b). In addition, aneuploid numbers ($2n = 43, 52$) and accessory chromosomes have been found by Neves (1952). In the Polish material however a karyological differentiation was not detected hitherto.

Iridaceae

Iris L. Studied by H. Wcisło.

Iris aphylla L. ssp. *bohemica* (Schmidt) Dostal ($2n = 48$).

In the Polish flora *I. aphylla* ssp. *bohemica* is a representative of the pontic element; it occurs only in a few isolated habitats on the Plateau of Little Poland. The plants for the present study have been collected in the steppe reservation "Biała Góra" near Tunel, district Miechów;

an isolated habitat of this ssp. has been found there by Medwecka-Kornaś (1953) in the steppe association *Inuletum ensifoliae*.

The chromosome number of *Iris aphylla* ssp. *bohemica* has not been reported hitherto. In the course of the present study the number $2n=48$ has been established. It is the same as that found previously by Pólya (1950) for ssp. *hungarica*. Other authors who studied this species (e.g. Simonet) did not give any information about the ssp. taken for their investigations.

Iris sibirica L. ($2n=28$).

This species is rather rare in the flora of Poland. Its area of distribution is restricted to the lowland where it grows only in swampy meadows. The plants studied originated from two habitats, in Pychowice and Kostrze, in the vicinity of Krakow. Their chromosome number $2n=28$ is in accordance with the results of previous authors: Kazao (1929, cit. from Tischler 1950) and Simonet (1932).

Thus, this species seems to be uniform in respect of its chromosome number.

Iris pseudacorus L. ($2n=24, 32, 34$).

This is the continuation of the author's investigation started some years ago. The first report (1957) concerned the occurrence of a cytotype with 24 somatic chromosomes in southern Poland. In addition to this number, plants with $2n=32$ and 34 have been detected in the course of the present work. The material collected hitherto originated from 40 natural habitats ranging from the Baltic coast in the north to the south of Poland (Leńcze near Kalwaria, N. lat. $49^{\circ}54'$). It has been pointed out previously (l. c.) that according to the results of other authors, this species manifests a well marked intraspecific differentiation. Further studies are in progress.

Gramineae

Poa L. ($x=7$). Studied by M. Skalińska.

Poa Chaixii Vill. ($2n=14$).

The occurrence of this grass in Poland is confined to mountains; in the Carpathian range it grows in plant communities consisting of tall grasses and herbs, on rather gentle slopes with a relatively high content of moisture in the soil. The plants for the present study originated from the range of Beskid Śląski near Żywiec (880 m c.s.l.) and from the range of Babia Góra (1020 to 1320 m o.s.l.) in the Western Carpathians (coll. Dr. S. Myszowski). The chromosome number $2n=14$ agrees with that previously reported by Stählin (1929) and by Avdulov (1931).

Poa bulbosa L. ($2n = 42$).

Our previous results concerning the chromosome number of this species in Poland (1957) have been supplemented by the study of plants collected in two further habitats: Grochów on the outskirts of Warsaw and Rembertów, north of Warsaw (coll. M. Piotrowicz). The two populations studied recently differ in respect of their chromosome number from our plants studied previously (aneuploids with 39 somatic chromosomes). In the two populations the hexaploid number ($2n = 42$) has been found. The same number has been reported by Hartung (1946) for specimens from North America.

Avenastrum (Koch) Jessen ($x = 7$). Studied by M. Skalińska.

Avenastrum planiculme (Schrad.) Opiz (*Avena planiculmis* Schrad.) ($2n = c. 120$).

This species is very rare in Poland. Its occurrence is confined to the mountains (Tatra Mts and the Sudetic Mts) where it is found in rare and isolated habitats. The plants for the present study have been collected by Dr. Z. Radwańska-Paryska on grassy slopes of Kominy Tylkowe in the Western Tatra at the altitude of about 1600 m.

A cytological study of this species has been performed by Reese (1953) on seedlings from the Botanical Garden in Göttingen; this study has revealed a chromosome number which is unusually high in grasses ($2n = c. 126$). Approximately the same number ($c. 120$) has been observed in root-tip mitoses of adult plants from the above mentioned natural habitat in the Western Tatra. It should be added that a similar chromosome number has been reported (evidently erroneously) by Katterman (in Tischler 1950) for the related species *Avena versicolor* (*Avenastrum versicolor*) of unknown origin. However a renewed study done on specimens from several natural habitats in the High Tatra led to establish for this species the diploid number ($2n = 14$) (Skalińska 1956).

Sieglingia Bernh. ($x = 6$). Studied by E. Banach-Pogan.

Sieglingia decumbens (L.) Bernh. ($2n = 36$).

This species occurs in barren meadows, in woods and in peatbogs. It is common in Poland both in the plain and in lower mountain regions. The specimens investigated have been collected in a pine wood near Maków Podhalański (foreland of the Carpathian range).

The chromosome number of this species ($2n = 36$) found in the Polish material agrees with the previous results of Wulff (1939) as well as those of Löve and Löve (1956). It should be added that according to Maude (1939) the chromosome number of this species is very high ($2n = 124$). This number however has not been confirmed hitherto by later studies.

Orchidaceae

Epipactis Zinn. em. Sw. Studied by E. Banach-Pogan and H. Weisło.

Epipactis latifolia (L.) All. ($2n=40$).

Epipactis latifolia represents an interesting example of intraspecific karyological differentiation ($2n=38$, Barber 1942, Hagerup 1947; $2n=40$, Hagerup 1947, Weijer 1952). In addition to the number $2n=38$ reported previously from Poland (Skalińska, Banach-Pogan, Weisło 1957), a further type, $2n=40$ has been found in the course of the present study in two habitats: road from Rabka to Poręba, in the Carpathian range, and Valley of Biały in the Western Tatra.

Listera R. Br. Studied by E. Banach-Pogan and H. Weisło.

Listera cordata (L.) R. Br. ($2n=38$).

This is a rare species in Poland occurring in shady woods chiefly in lower mountain habitats. The plant material originated from three habitats in the Tatra Mts: the slope of Kopieniec (c. 1300 m o.s.l.), the common "Polana pod Wołoszynem" (c. 1254 m o.s.l.) and the Valley of Rożtoka near the cascades "Wodogrzmoty Mickiewicza" (c. 970 m o.s.l.). In all plants studied only the number $2n=38$ has been found. A detailed study of the chromosome morphology led to establish the occurrence of two pairs of long bi-armed chromosomes, one pair of somewhat smaller chromosomes and 16 pairs of small ones (Fig. 34). The somatic number 38 agrees with that given by Sokolovskaja and Strelkova (1940, cited from Tischler 1950), while neither the number 42 reported by Harding (in Tischler L. C.) nor any deviation towards the lower number 36 observed by Löve and Löve in specimens from Iceland (1956), could be found in the material from Poland.

SUMMARY

The present study deals with the karyology of 43 species of Dicotyledons and Monocotyledons from natural habitats in Poland, compared with the results obtained by previous authors for plants from other geographic areas.

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An interference-filter monochromator system for the irradiation of microscopic objects

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INTRODUCTION

In photobiological experiments interference filters are widely used for the effective isolation of narrow spectral regions. Withrow (1957) and Mohr and Schoser (1959, 1960) have recently described equipments allowing the irradiation of experimental objects with monochromatic light of relatively high intensity, i.e. of the order of some thousand ergs per cm^2 sec., obtained from interference filters in the visible and near-visible range. Cyto-physiological researches undertaken in this laboratory have necessitate the construction of a similar equipment allowing the irradiation of microscopic objects with monochromatic light of very high intensity, of the order of some tens of thousands ergs per cm^2 sec. The present report describes this equipment and the method of measuring light intensity.

DESCRIPTION OF THE APPARATUS

The apparatus consists of ten identical units each composed of a projection lamp, a collector lens, an aqueous filter, an interference filter, a microscope mirror, and a condenser. The units are arranged side by side on a common bench. The lamps have one common housing and the condensers are mounted side by side on a common stage more than 100 cm. long. The microscope tube travels on a rail above the stage and is provided with a spring catch which allows to set it in line with the optical axis of each unit. This equipment makes possible the simultaneous irradiation of 10 microscopic objects with monochromatic light of different wave lengths and the successive microscopic observation of the objects (Fig. 1 and 2).

The sources of light are 100 W or 750 W — according to the desired light intensity — incandescent lamps with tungsten biplane filaments and a nominal voltage 110 V. The lamps are operated with 100 V current from three variable transformers, each transformer feeding three or four units.

A voltmeter connected to each set of units allows the control of the voltage. The socket of each bulb is so constructed that its position can be adjusted in three directions. All the lamps are housed under a common cover of aluminium sheet lined inside with asbestos fabric. When 100 W lamps are used no cooling is necessary, but with the 750 W ones 10 centrifugal air blowers must be started to cool the bulbs.

The optical system of each unit is fixed on a common rail and consists of a double lens, each $f = 6,7 \text{ cm}$ ($+ 15 \text{ D}$), a diafragma placed between the lenses for limiting the light field, a plane glass cell for the aqueous filter of path length 5 cm., a lens of focal length 10 cm, and a microscope

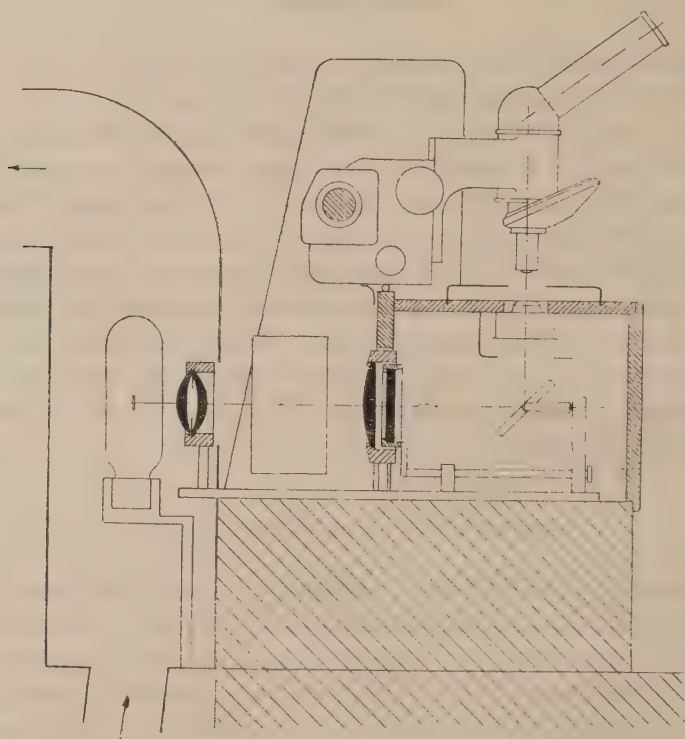


Fig. 1. Cross-section view of the apparatus for the irradiation of microscopic objects (for details see text)

mirror. Directly behind the second lens there is the interference filter mounted in a way allowing its easy and rapid removal from the path of the light. The mirror directs the light beam upwards onto the microscope condenser (N. A. 1,2) composed of two lenses and mounted in the microscope stage. Below the stage there is a sliding frame for the neutral filters used to reduce the light intensity. Fig. 3 shows the dimensions of the optical system and the path of light rays. As is to be seen the lenses

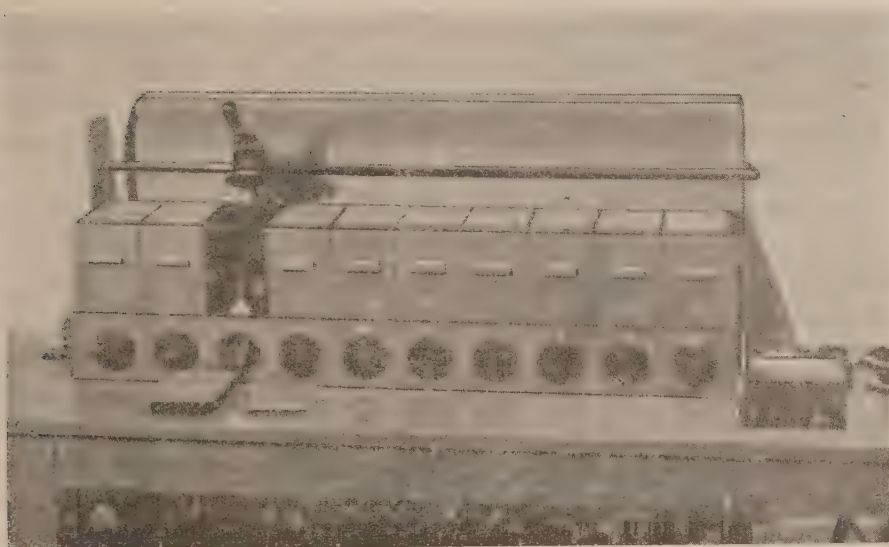


Fig. 2. General view of the apparatus

are arranged according to the Kohler's system which gives a uniform distribution of light intensity in the light spot when the system is properly regulated. Measurements with a photomultiplier of the light intensity distribution in the image of the field of vision of the microscope have shown that the local differences do not exceed ± 10 per cent. The diameter of the lighted field in this equipment is 2 mm. when the diafragma aperture diameter is 16 mm. and can be increased by widening the diafragma aperture.

The microscope stage is of duralumin and its under side is provided with a copper tube spiral connected to an ultrathermostat for heating or

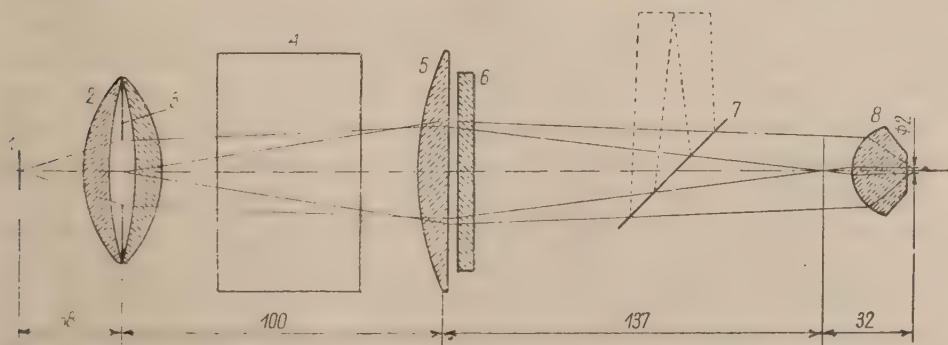


Fig. 3. The optical system: 1 — filament of bulb; 2 — lens $f = 6,7$ cm. diameter 45 mm.; 3 — diafragma diameter 16 mm.; 4 — water filter; 5 — lens $f = 10$ cm. diameter 58 mm.; 6 — interference filter diameter 50 mm.; 7 — mirror; 8 condenser

cooling the stage. The microscopic slides are held in position on the stage with clips. Each slide is covered by a lid of sheet metal sliding in grooves cut in the microscope stage which make the lids absolutely lightproof.

The top part of the microscope (type M-110, PZO, Warszawa), consisting of an inclined monocular tube, a revolving nosepiece with $10\times$ and $40\times$ objective lenses, an eyepiece, and a device for coarse and fine adjustment, travels along the microscope stage. The top part of the microscope is provided with a spring catch device allowing to move the microscope from one unit to another within a few second.

The air-cooling of strong lamps may cause vibrations transferred from the rotors and blowers to the apparatus, and this may impede observations especially when larger magnification are used. However, the vibrations can be almost completely eliminated by placing the blowers on a separate base and connecting them to the lamp housing through wide rubber tubes.

THE EFFICIENCY OF THE OPTICAL SYSTEM

Single line interference Zeiss (type JF) and Schott (type IL) filters ranging from 400 to 750 $m\mu$ are used. Their optical characteristics have been described by various workers, e.g. Günzler (1956), and Mohr and Schoser (1959). The heat filters consist of the following water solutions: $CuSO_4$ 100 g/l with 2 per cent addition of sulfuric acid for the wave length range 400—480 $m\mu$, $Fe(NH_4)_2(SO_4)_2$ 70 g/l with 1 per cent addition of sulphuric acid for the wave length range 490—670 $m\mu$, and pure water for the wave length range 680—750 $m\mu$ (Withrow and Price 1956).

Table 1

Filter	Maximum light intensity with bulb of	
	100 W	750 W
400 IL	3 100 ergs/cm ² sec	14 700 ergs/cm ² sec
442 IL	10 800 "	63 500 "
541 IL	23 100 "	86 400 "
662 IL	22 600 "	92 000 "
713 JF	28 200 "	108 000 "

With this set of filters the intensity of the irradiation of the microscope objects ranges from several thousand to several tens of thousands ergs per cm² per sec. according to the wave length and the power of the lamps. Some examples of maximum light intensity are shown in table 1.

Table 2

	Diameter of illuminated surface	Maximum light intensity in ergs/cm ² sec.	
		about 410 m μ	about 620 m μ
Withrow (1957) lamp of 750 W	20 cm	200	1 000
Withrow (1957) carbon arc	20 cm	—	20 000
Mohr and Schoser (1959) lamp of 750 W	5 cm	1 500	14 000
Mohr and Schoser (1960) xenon arc	9 cm	4 700	6 300
Apparatus described in this paper			
lamp of 100 W	2 mm	4 000	22 000
lamp of 750 W	2 mm	15 000	90 000

Since the light intensity changes as the lamps are used up measurements must be carried out after keeping a lamp alight for 6—10 hours.

The direct comparison of the apparatus here described with similar equipment constructed by Withrow and by Mohr and Schoser is difficult because those workers designed their equipment for irradiating larger surfaces. The approximate maximum intensities of blue and red light as well as the diameters of the illuminated surfaces in different types of monochromators are compared in table 2.

MEASUREMENTS OF LIGHT INTENSITY

For measurements of absolute light intensity on the small lighted area of the microscopic slide a specially designed thermocouple was used. It consisted of a manganin-constantan strip 7 μ thick, 0.16 mm wide and 7 mm. long stretched between two copper wires 0.5 mm. in diameter. The attachments of the strip to the wires were carefully soldered with Wood metal and the strip was coated with lamp black. The thermocouple was mounted on a slide glass 1 mm. thick in such a way that the strip was 0.3 mm. above the surface of the glass. The details of the thermocouple design are shown in figs. 4 and 5. The resistance of the thermocouple together with about one meter of conduits was 5.2 Ω . The thermocouple was calibrated according to a Kipp and Zonen type El thermopile of known absolute sensitivity. The sensitivity of the thermocouple when its whole length was irradiated was 9.28 μ V/mW. However, during the measurements of the intensity of the light spot only a part of the strip was illuminated and thus it was necessary to examine the sensitivity of the various segments of the thermocouple. For this purpose the thermo-

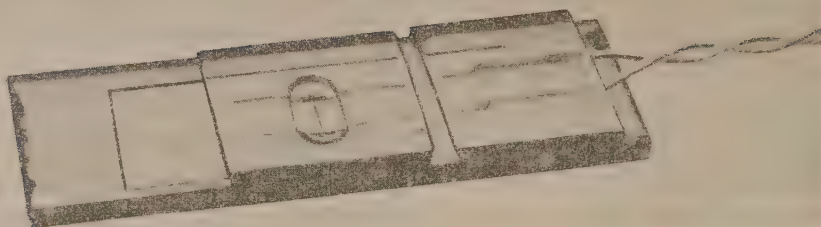


Fig. 4. Thermocouple for measurements of absolute light intensity

couple was moved over a narrow slit of light (obtained in a microscope by applying a slit diafragm in the Köhler system) and from the deflections of the galvanometer the point of the greatest sensitivity of the thermocouple was found (Fig. 6 a). Then the thermocouple was placed with the point of the greatest sensitivity over the centre of the light slit and by altering the width of the slit the length of the illuminated thermocouple segment was changed at constant intensity of the incident light; the

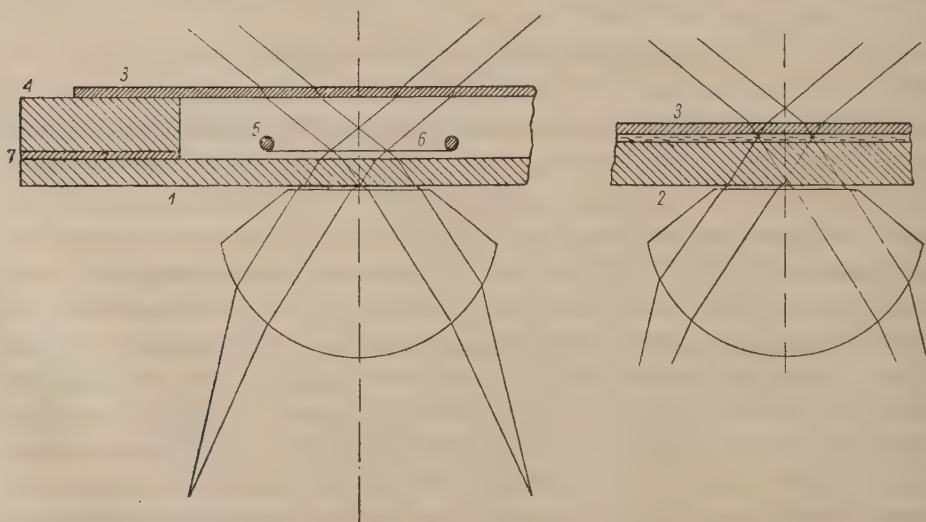


Fig. 5. Cross-section of thermocouple (left) and cross section of microscopic object (right) with marked paths of light rays. 1 — glass slide 1 mm. thick; 2 — glass slide 1,5 mm. thick; 3 — cover glass; 4 — plexiglass plate 2 mm. thick with imbedded copper wires; 5 — copper wires diameter 0,5 mm.; 6 — thermostrip; 7 — plate 0,3 mm. thick

galvanometer recordings showed the dependence of the thermocouple potential on the size of the light spot (Fig. 6 b). The standard curve plotted from a series of such recordings was used for establishing the corrections to be used in calculations of the absolute light intensity. E.g. in the case of a light spot 2 mm. in diameter applied in the apparatus here described the thermoelectric potential was 68 per cent of the potential which would have been obtained if the strip was irradiated over its whole length with the light of the same intensity.

The wiring system of the thermocouple and the galvanometer was for every recording the same as the circuit described by Kok (1948) for the calibration of galvanometers. The magnitude of the resistance applied

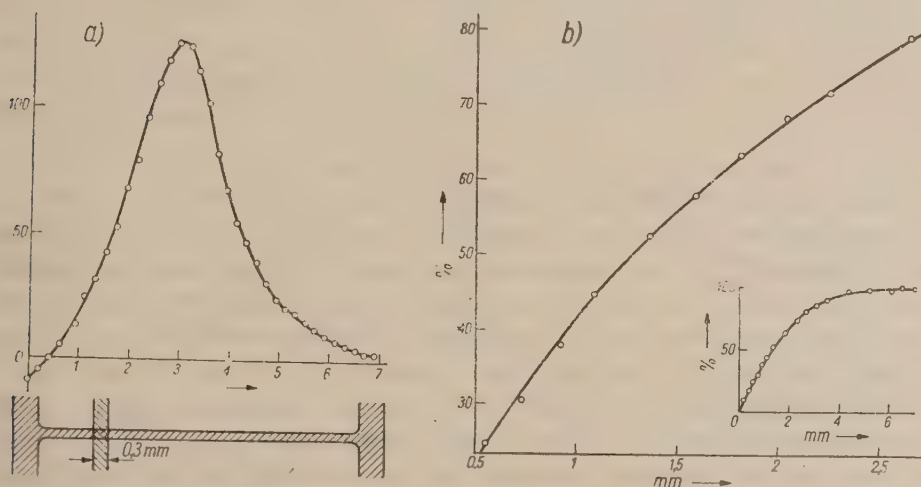


Fig. 6. The local sensitivity of the thermocouple: *a* — galvanometer deflection according to the position of light beam 0.3 mm. wide, *b* — dependence of the thermoelectric potential on the width of the light spot when the centre of the light spot coincides with the point of maximum sensitivity of the thermocouple strip

in the voltage regulator was known to within 0.01 per cent. The following galvanometers were used: a Kipp and Zonen portable galvanometer type A 70 of internal resistance 80 Ω , and a Zeiss string galvanometer of internal resistance 5.5 Ω .

The absolute light intensity was calculated from the formula:

$$W = x \frac{V \cdot r}{z \cdot y \cdot s}$$

where W is the absolute light intensity in mW/cm^2 ,

x is the mean galvanometer deflection after switching on the light,

z is the mean galvanometer deflection after switching on the micro-volt potentiometer,

V is the voltage of the element of the voltage regulator in μV .,

r is the ratio of the voltage distribution R_0/R_x

y is the absolute sensitivity of the thermocouple in $\mu V/mV$.

s is the lit area coefficient of the thermocouple.

Here is an example of actual measurements:

Filter 622 $m\mu$, 100 W lamp, A 70 galvanometer, mean deflection after switching on the light $x = 19,42$, mean deflection of the galvanometer $z = 44,04$ when the voltage element was $V = 1,58 V$ and the arrangement of the resistance was such that the ratio of the voltage distribution was $r = 1,77 \times 10^{-5}$. The absolute sensitivity of the thermocouple was $9,28 \mu V/mW$ and the surface coefficient for the light spot 2 mm. in diameter was $s = 0,68$. Therefore,

$$W = 19,42 \frac{1,58 \cdot 10^6 \cdot 1,77 \cdot 10^{-5}}{44,04 \cdot 9,28 \cdot 0,68} = 2,074 \text{ mW/cm}^2.$$

Thus the absolute light intensity was 20 740 ergs per cm^2 . sec.

The question still remains whether the light intensity in the plane of the microscopic objects is the same as the light intensity of the light spot measured with the thermocouple. As is to be seen from the diagram (fig. 5) in both cases the path of light rays is exactly the same till the beam penetrates the slide. In the case of the thermocouple light passes through one mm. of glass and than through air, whereas in the case of the microscopic object the thickness of glass is 1,5 mm. and water is the medium surrounding the object. Thus in each case there is a difference of absorption in the glass and of reflection caused by the different media (glass-air and glass-water). In view of the very small absorption of light in glass in the 400—2000 $m\mu$ wave lenght range the difference of 0,5 mm. in the thickness of slides may be disregarded. However, in the two cases the losses of light intensity caused by reflection differ significantly. When the incident light rays are normal to the boundary between the two media the theoretically calculated losses caused by reflection are 0,425 per cent when light passes from glass ($n = 1,52$) to water ($n = 1,33$) and 4,25 per cent when light passes from glass to air ($n = 1$). In a beam of light passing through a condenser of given aperture not all the light rays are normal to the boundary, but on the contrary most rays strike the boundary at different angles. The reflection of rays striking the boundary obliquely may be much greater, especially if the angle of incidence approaches the critical angle. The drop of light intensity according to the angle of incidence of light rays calculated theoretically from Fresnel's laws of reflection (Jenkins and White 1957) is illustrated by the

curve in fig. 7. As is to be seen from the graph the difference of reflection between the glass-water boundary and the glass-air boundary increases markedly only when the angle of incidence of the beam is greater than $30\text{--}35^\circ$. In the beam of light here considered rays striking the boundary at this angle occur only when the aperture is more than 0,8, and even then their proportion is small. In order to measure experimentally how the difference of reflection depends on the aperture number a series of observation was carried out on two different microscopic objects: 1) glass slide 1,5 mm. thick, water and cover glass, and 2) glass slide 1 mm. thick, air and cover glass. The intensity of transmitted light was measured in a beam of parallel rays and for different apertures. The light was monochromatic of the wave length $546\text{ m}\mu$. The objectives used for these experiment always had a larger aperture than the apertures used in the illuminating system and were always set to the sharpness plane of the light spot (in the object consisting of glass and water the light spot is situated about 0,2 mm. higher than in the object consisting

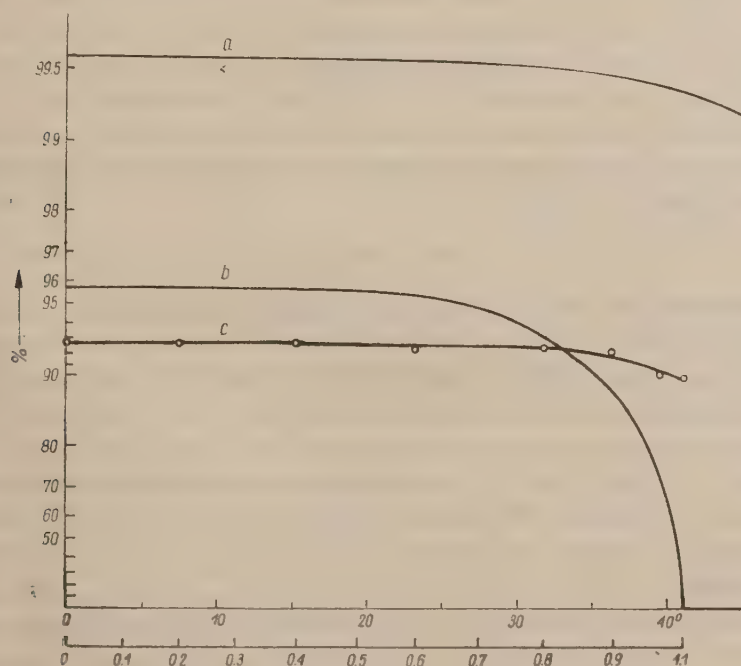


Fig. 7. Reduction of light intensity caused by reflection: *a* — theoretical for glass-water boundary plotted against incidence angle, *b* — theoretical for glass-air boundary plotted against incidence angle, *c* — experimental for two glass-air boundaries as compared with two glass-water boundaries (assumed as 100 per cent) plotted against aperture

of glass and air). The light detector was a photo-element placed over the objective in the plane in which the image is formed and connected to a Zeiss scale galvanometer. The loss of light intensity caused by reflection was in this case twice as large as in measurements of absolute light intensity because the light rays passed twice through the boundaries between different media (glass-water and glass-air). The results of the measurements are shown on the graph in fig 7. The drop of intensity in a normal beam of parallel rays was in accordance with the theoretical calculations. The intensity of a beam of light passed through a dry object was 92,76 per cent as compared to the intensity of a beam passed through the other object taken as 100 per cent. When the beam was composed of convergent rays the light intensity decreased only slightly, dropping to 39,5 per cent when the aperture was 1,0. The reason for such a small decrease was that even in the case of aperture approaching 1,0 the rays striking the boundary at angles close to the critical angle amounted to only a small fraction of the whole beam.

From the above results it has been accepted that the intensity of light for a microscopic object (water medium) is 4 per cent higher than the intensity measured with the thermocouple. This correction has been established for the 0,8 condenser aperture but without major error it is valid for apertures ranging 0,5 to 1,0 (actually the correction is 3,35 per cent for a beam of parallel rays and 5,03 per cent for an aperture 1,0).

Thus in the above quoted example after accounting for reflection the measured intensity of light is:

$$W' = W \cdot 1,04 = 2074 \cdot 1,04 = 21\,560 \text{ ergs/cm}^2 \text{ sec.}$$

A thermocouple similar to the one described here was also constructed by Manton and Milatz (1953). However, the strip of their thermocouple was of lesser width, thinner and was placed in a vacuum chamber which improved sensitivity. The advantages of the thermocouple here described are the simple construction and the possibility of placing the strip exactly in the plane of the measured light spot which is obtained by microscopic control. The sensitivity of the thermocouple is sufficient to measure the absolute, maximum light intensity with an error of less than 5 per cent.

The absolute measurements of light intensity are made only for the maximum intensities. The lower intensities obtained by the interposition of neutral filters are measured relatively to the maximum intensity. For this purpose a photomultiplier with a RCA 931A cell is used. The cell is placed over the microscope eyepiece in the plane of the field of vision. The set of neutral filters makes possible the reduction of light intensity to any desired level.

SUMMARY

The monochromator apparatus with interference filters is used for irradiation of microscopic objects. The apparatus allows the simultaneous irradiation of 10 slides with monochromatic light within the range 400 to 750 m μ . The diameter of the irradiated field is 2 mm. The maximum intensity when single line filters are used ranges from 15 000 ergs/cm²sec. for 400 m μ to 100 000 ergs/cm²sec. for 700 m μ .

A method is described for measuring the absolute light intensity when the illuminated surface has a small area. The method consists in the application of a specially constructed thermocouple allowing measurements of light intensity of a beam of light 0.3 to 6 mm. in diameter. The sources of errors arising in the measurements are analysed.

Acknowledgements

The author's most sincere gratitude is due to Doctor B. K $\ddot{o}$ k for his valuable advice on the methods of light intensity measurements in small surfaces, to Kipp and Zonen for supplying the thermostrips used in the thermocouple and to the PZO Works in Warsaw for supplying the microscope parts necessary to construct the apparatus.

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The influence of chloroplast displacements on the optical properties of leaves

by

J. ZURZYCKI

INTRODUCTION

Changes in the arrangement of chloroplasts in leaf cells alter the optical properties of leaves, e.g. the profile arrangement assumed by chloroplasts under the influence of strong light leads to a drop in the amount of absorbed light energy. As early as 1880 Stahl making direct prints of leaves on photographic paper demonstrated that the parts of leaves previously exposed to strong light transmitted more of the photochemically active light rays and gave darker prints than the parts not exposed to light. Babuskin (1955), using the same method for determining the action spectrum of phototaxis, assumed that the density of sensitized paper after exposure was a criterion of chloroplast arrangement. Recently Biebl (1954, 1955) applied a photovoltaic cells placed directly under a leaf exposed to light for measurements of light transmission. In many instances the light transmission of leaves changed according to expectation in view of the changes of the chloroplast arrangements. However, in the leaves of some species there was no clear dependence between chloroplast arrangements and transmission or the dependence was directly opposite to what was expected. E.g. the light transmission of *Sambucus nigra* leaves was lower after exposure to strong light when measured with the photovoltaic cell, though with Stahl's method of direct prints on photographic paper a higher transmission of these leaves was observed (Biebl 1955). Is this result interpreted as caused by the difference in the spectral sensitivity of the two detectors?

Only a few of the investigations just mentioned were connected with the microscopical control of the chloroplast arrangements (Biebl 1954). and thus as was rightly stressed by Seybold they usually could not precisely define what was the influence of the chloroplast arrangements on the transmission of leaves. Similarly little is known of the actual changes in the absorption of light energy that take place after the chloro-

plasts are regrouped. Theoretical consideration lead to the conclusion that changes of this kind must take place, but their magnitude is not very great (Zurzycki 1953, Seybold 1955). However some simplifications and not always verifiable assumptions have to be of necessity introduced into the theoretical calculations and accurate data on the actual changes in the amount of absorbed energy may only be obtained from experimental spectrophotometric measurements.

Some information on this problem is contained in the reports of Detlefson (1888) as well as of Schanderl and Kaempfert (1933). Detlefson measured with a thermocouple the transmission of illuminated leaves of *Humulus lupulus* and found that transmission increased by 12 per cent during 37 minutes. He considered the displacement of chloroplasts to be a possible cause of this change. Working with leaves of *Tradescantia viridis* Schanderl and Kaempfert found that after exposure to sun light transmission increased from 6 to 12 per cent in the yellow-green part of the spectrum and from 3 to 10 per cent in the blue-violet range. Seybold, who doubts the accuracy of these results (1955), has been the first to study the full characteristic of changes in the optical properties of leaves (1956). Using a Hardy spectrophotometer he determined the changes of spectral reflection, transmission and absorption of leaves of *Begonia multiflora*. He found that absorption was distinctly altered, but not more than 5 per cent, after the leaves were exposed to strong light. It seems that in the thin and more translucent leaves of water plants, which are usually used for experiments on the displacement of chloroplasts, the changes of absorption should be somewhat greater. The investigations here reported were carried out on the leaves of water plants and were intended to supply an answer to the following questions:

1. Did a simple measurement with a photovoltaic cell of light transmission of leaves having a single or a few layers of cells correlate with the chloroplast arrangement and could it be regarded as indicative of the chloroplasts arrangements?
2. How did the actual optical conditions of leaves change for various chloroplasts arrangements?

METHOD

The measurements were carried out on fronds of *Lemma trisulca* and on leaves of *Funaria hygrometrica*, *Elodea canadensis* and *Fontinalis antipyretica*. In the leaves of the last of these species there are no chloroplast movement and the species was used for obtaining comparative data.

The optical properties of all these species could be determined on

a small surface ranging 0,5 to 2 mm. in diameter. The simple method of measuring light transmission consisted in measurements of light intensity with a photovoltaic cell in the plane of the microscope image. The microscope was of the kind designed for microphotography with a side eyepiece. The photovoltaic cell was placed in the plane of the photographic plate and the arrangement of chloroplasts was observed simultaneously through the side eyepiece. The microscope used for these measurements was equipped with a $10\times$ objective and a $7\times$ eyepiece. The light was white or blue (filter BG 23—2 mm). The diameter of the lighted field and at the same time the field of vision was 0,92 mm.

Being translucent but not transparent leaves reflect and transmit light differently than solution. The spectrometric techniques for that kind of biological objects was developed by Shibata (1958, 1959) and

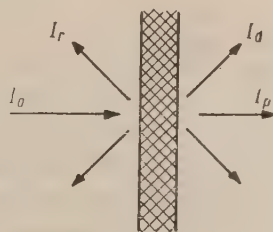


Fig. 1. Path of light in translucent materials (leaves).
 I_0 — incident light, I_p — parallel transmitted light, I_d —
diffuse transmitted light, I_r — reflected light

his method was applied in the present work. Shibata's terminology was supplemented by the concept of translucance; the logarithm of the reciprocal of translucance corresponds to Shibata's attenuance. For the sake of precision the various values determined in the experiments are listed and defined below, where I_0 is the intensity of incident light, I_r the intensity of reflected light, I_p the intensity of parallel transmitted light and I_d the intensity of diffuse transmitted light (Fig. 1).

$$\frac{I_p}{I_0} = \text{rectilinear translucance}$$

$$\frac{I_p + fI_d}{I_0} = \text{quasi translucance}$$

$$\frac{I_p + I_d}{I_0} = \text{semi-integral translucance}$$

$$\frac{I_p + I_d + I_r}{I_0} = \text{integral translucance}$$

$$\frac{I_r}{I_0} = \text{reflectance}$$

$$1 - \frac{I_p + I_d + I_r}{I_0} = \text{absorptance}$$

$$\lg \frac{I_0}{I_p} = \text{rectilinear attenuation}$$

$$\lg \frac{I_0}{I_p + f I_d} = \text{quasi attenuation}$$

$$\lg \frac{I_0}{I_p + I_d} = \text{semi-integral attenuation}$$

$$\lg \frac{I_0}{I_p + I_d + I_r} = \text{integral attenuation}$$

$$\lg \frac{I_0}{I_r} = \text{reflex attenuation}$$

$$\frac{f_\alpha I_r}{I_0} = \text{angular reflection}$$

$$\frac{f_\alpha I_d}{I_0} = \text{angular dispersion}$$

The measurement of the intensity of light transmitted by an object with a photovoltaic cell fitted to a microscope in the way described above gives the value of quasi transmittance, where f depends on the optical system. In the case of the $10 \times$ (N. A. 0,24) objective used in the experiments the light detector was reached by rays dispersed by not more than 14 degrees.

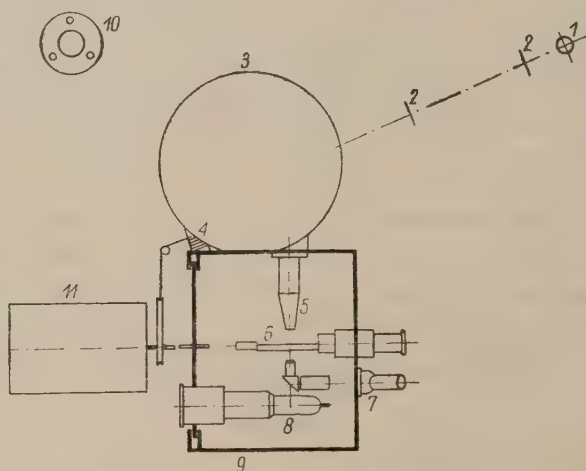


Fig. 2. Schematic diagram of apparatus. 1 — 250 W projection lamp; 2 — light focusing optical system; 3 — mirror monochromator; 4 — monochromator drum; 5 — optical system focusing monochromatic light; 6 — stage for holding the object; 7 — control microscope; 8 — photomultiplier; 9 — chamber in which observations are made; 10 — galvanometer; 11 — optical kymograph

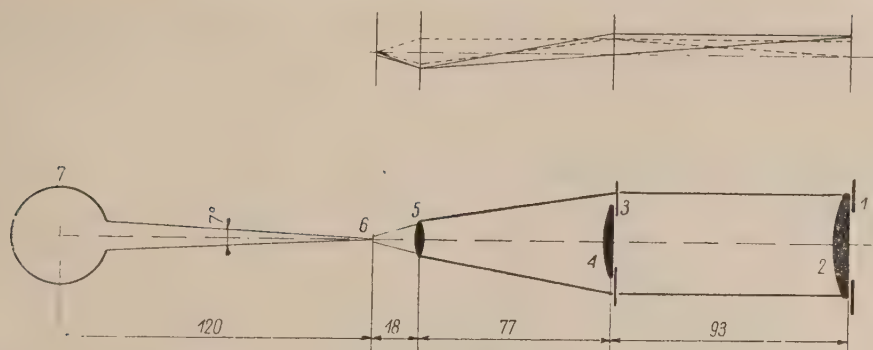


Fig. 3. Optical system focusing monochromatic light. 1 — aperture of monochromator; 2 — lens $f = 7,15$ cm.; 3 — iris diaphragm, 4 — lens $f = 4,55$ cm.; 5 — lens $f = 1,2$ cm.; 6 — light spot; 7 — photomultiplier. Dimensions in the diagram in millimeters

The absolute measurements of the other optical properties were made in a special apparatus utilizing Shibata's opal plate principle. The design of this apparatus is shown in fig. 2. The source of light was a 250 W lamp supplied from a stabilizer; the light condensed by a system of lenses reached the entrance slit of a Zeiss mirror monochromator; the light rays coming out from the monochromator were condensed by an optical system shown in fig. 3 and gave an uniformly illuminated light spot 2.5 mm. in diameter. The size of the light spot could be freely reduced by the iris diaphragm incorporated in the optical system. The aperture of the lighting system was about 0.25. The object i.e. the microscopic sample on an opal slide held on a special stage, was placed in the plane of the light spot. The stage with the slide held on it could be moved and fixed in three positions for making zero and blank measurements. A system of levers working on the principle of a cross stage made possible the placing of any part of a leaf in the light beam. An auxiliary microscope with a low magnification objective was used for the control of the part of the sample exposed to light. The details of the arrangement are shown in fig. 4. The optical system directing the light from the monochromator, the microscopic stage and the objective of the control microscope were placed in a lightproof box in which the preparations were irradiated. The light detector, i.e. a Zeiss M 12 S photomultiplier, also placed in the box could be adjusted at various angles ranging from 0 to 150 degrees with regard to the beam of light. This equipment allowed the measurement of the following optical properties of leaves:

1. Rectilinear transmittance — the leaf was mounted on a transparent slide and the detector was set at 0 degrees, i.e. the axis of the light beam (the angle of the light beam that reached the photomultiplier being

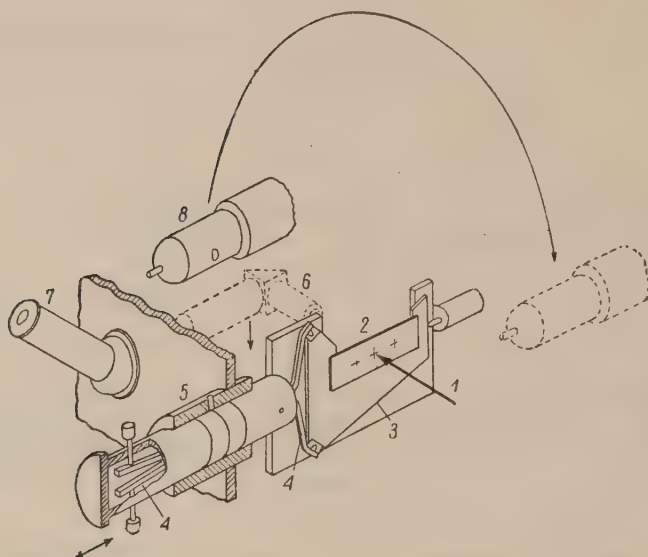


Fig. 4. Stage for holding object and the control microscope. 1 — path of the light beam; 2 — slide; 3 — movable stage adjusted with levers 4, 5 — tube with spring catch; 6 — prism and microscope objective (can be shifted downwards); 7 — tube of microscope; 8 — photomultiplier

7 degrees the light rays may be regarded as almost parallel). The reference light intensity was the light transmitted by the slide with no leaf.

2. Angular dispersion and reflection — sample on transparent slide, detector adjusted at various angles ranging 0 to 150 degrees the reference light intensity same as in point 1.

3. Semi integral translucance without correction for multiple reflection — sample on opal slide, detector in the 0 degrees position, the reference light intensity same as in point 1.

4. Reflectance — preparation behind the opal slide, detector in the 150 position, the reference light intensities were black paper on slide and an additional layer of opal glass (Shibata 1958).

The values of reflectance and semi integral translucance have been calculated from the following formulae:

Reflectance:

$$R_s = \frac{1}{R_1 + \frac{D_2}{D_{1+s}} \frac{1}{R_1} + R_1} \quad (\text{Shibata 1958, formula 14})$$

where D_2 is the difference between the galvanometer deflections for light reflected from two opal plate and from one plate with black paper,

D_{1+s} is the difference between the galvanometer deflections for light reflected from a plate with the object and from a plate with black paper, and

R_1 is the constant characteristic for the plate.

The value of R_1 was 0.467 and was determined according to Shibata's (1958) method, the only difference being that instead of grey film a neutral filter NG 5.2 mm. was used.

Semi integral transmittance:

$$T_s = \frac{1 - R_1 R_s}{K_1 / K_{1+s}} \quad (\text{deduced from Shibata's formula 7})$$

where T_s is semi integral transmittance corrected for multiple reflection:

$$\frac{I_p + I_d}{I_0},$$

K_1 is the deflection of the galvanometer for light passing through opal glass,

K_{1+s} is the deflection of the galvanometer for light passing through opal glass and the sample.

Absorptance:

$$A_s = 1 - (R_s + T_s).$$

The output current of the photomultiplier was measured with a rapid response galvanometer of inner resistance 680 Ω , oscillation period 1.5 sec. and sensitivity $4 \cdot 10^{-10}$ A/mm. m. The deflections of the galvanometer were recorded on an optical kymograph. The axis of the kymograph could be coupled either with the angular gear of the photocell — the graph that illustrated the angular dispersion — or with the drum of the monochromator and then the graph represented the galvanometer deflection with regard to the wave length. An additional lamp lit by suitable connections on the monochromator drum marked light lines on the kymograph drum and these lines were the datum points for the scale of wave lengths. From the graphs thus obtained the values of R_s , T_s and A_s were calculated in the manner already described at intervals of 5 or 10 m μ for the scale of wave lengths and at intervals of 5 degrees for the angular scale. The recording of the leaf spectrum could be complete during a relatively short time, i.e. 8 to 10 minutes, which was important in so far as the chloroplast arrangement obtained in the initial exposure to light was not stable and changed in the dark chamber in which the measurements were made. However, during 10 minutes the changes of the chloroplast arrangement were on the whole insignificant.

RESULTS

I

In the part of the fronds of *Lemna trisulca* with a single mesophyll layer as well as in the leaves of the other species used in the experiments the value of quasi transluctance for the flat arrangement of chloroplasts was 30 to 60 per cent. Under stabilized light conditions — in weak light — the value of quasi transluctance changed but little, the range of the changes not exceeding 2 per cent. After the chloroplasts had been rearranged into the profile position (strong light) quasi transluctance increased by 8 to 20 per cent. The use of the blue filter increased the recorded differences up to 30 per cent.

Table 1

Quasi transluctance of leaves for the flat and profile arrangements of chloroplasts

Object	Light	Quasi transluctance in epistrophe %	Quasi transluctance in parastrophe %	Increment %	Relative increase in relation to epistrophe = 100%
<i>Lemna trisulca</i>	white	45.9	51.8	5.9	12.86
<i>Lemna trisulca</i>	white	44.4	52.2	7.8	17.56
<i>Lemna trisulca</i>	blue	36.8	46.8	10.0	27.38
<i>Lemna trisulca</i>	blue	47.5	56.9	9.4	19.77
<i>Lemna trisulca</i>	blue	35.5	43.9	8.4	23.66
<i>Funaria hygrometrica</i>	blue	58.3	65.0	6.7	11.50
<i>Funaria hygrometrica</i>	blue	62.0	67.3	5.3	8.55
<i>Elodea canadensis</i>	blue	30.2	40.1	9.9	32.75

Some values of quasi transluctance obtained in various experiments and for leaves of various species are assembled in table 1. The differences of the values of quasi transluctance and of the changes in this value when the chloroplasts passed into the profile arrangement were rather high even within one species. These differences were comprehensible in view of the considerable individual differences in the anatomic structure of leaves and chloroplasts, the concentration of pigments in the plastids etc.

In order to check whether the changes of quasi transluctance conformed with the course of the chloroplast rearrangement measurements were carried out for recording simultaneously in the course of a phototactic reaction the percentage of epistrophe — established by counting the number of chloroplasts in the flat arrangement — and the changes of

quasi transmittance (Figs. 5—9). The course of a typical reaction of this kind is shown in fig. 5. A change in the number of chloroplasts in the flat arrangement corresponded to the increase of quasi transmittance. When all the chloroplasts assumed the profile arrangement quasi transmittance increased from the initial value of 48.8 to 56.7 per cent (i.e. by 16.18 per cent) in white light. In blue light the increase was from 41 to

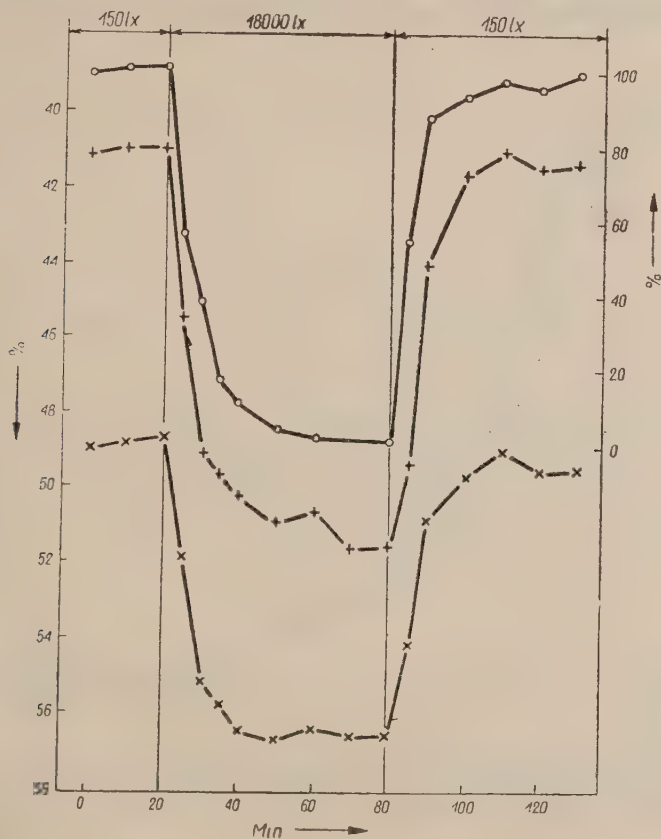


Fig 5. *Lemna trisulca*. Changes in the chloroplast arrangement (right scale) and of quasi transmittance (left scale) — o — percentage of epistrophe; — x — quasi transmittance in white light; — + — quasi transmittance in blue light. X-axis — time in minutes. The figures above indicate the intensity of light

51.7 per cent, i.e. by 26.21 per cent. The return of chloroplasts to the flat arrangement was accompanied by a drop of quasi transmittance to approximately the original value. The changes in the value of quasi transmittance and in the arrangement of chloroplasts as determined numerically are distinctly parallel. This conformity was only disturbed in the first stage of the phototactic reaction the increase of transmittance at first

being slower than the decrease of the number of chloroplasts in the flat arrangement. The lack of conformity was better brought out by a series of measurements carried out at shorter intervals (Fig. 6). The certain disagreement between the two methods of measurement is easy to explain. In the initial stage of reaction the part of chloroplasts which had moved to the side cell walls markedly decreased the percentage of epistrophe, but in the cell they occupied spaces where previously there were no chloroplasts and, though their individual absorption diminished

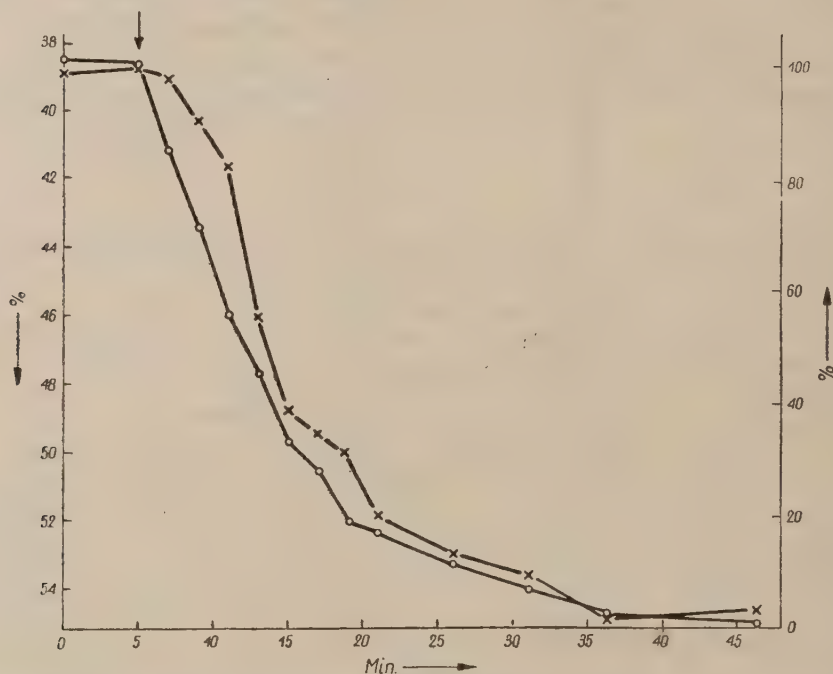


Fig. 6. *Lemna trisulca*. Changes of the chloroplast arrangement and of quasi transmittance recorded in blue light. From the fifth minute the intensity of light was 30 000 luxes

owing to their different orientation with regard to the direction of light (Zurzycki 1953), the change was relatively small and only slightly influenced the overall absorption of the leaf. It was not till the later stages of the reaction when the chloroplasts were arranged in two or more layers by the side cell walls that the change of absorption was more distinct.

Investigations on the changes of light transmission of terrestrial plants in very strong light have shown that after the initial increase transmission sometimes decreased (Schanderl and Kaempert 1934, Biebl 1954). This effect was usually regarded as being caused either by the

accumulation of assimilating starch or by changes in the water content of leaves. In water plants the latter of these possibilities seems out of the question. To check whether also in water plants transmission would

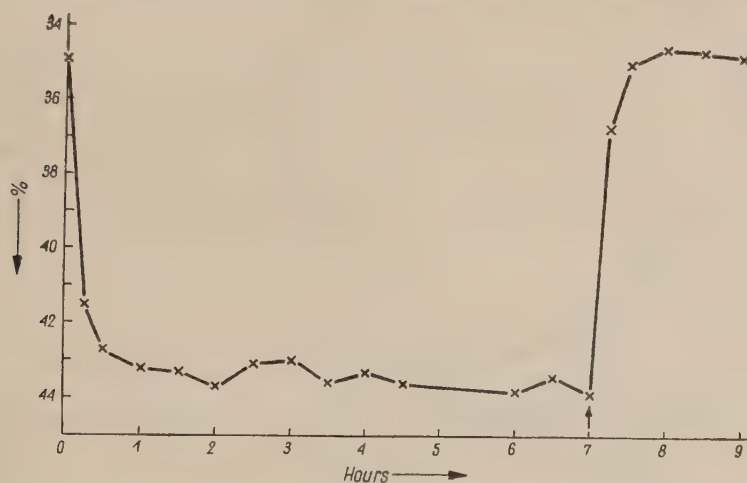


Fig. 7. *Lemna trisulca*. Changes of quasi transluance recorded in blue light during 7 hours of exposure to light of 30 000 luxes. X-axis — time in hours; Y-axis — transluance percentage

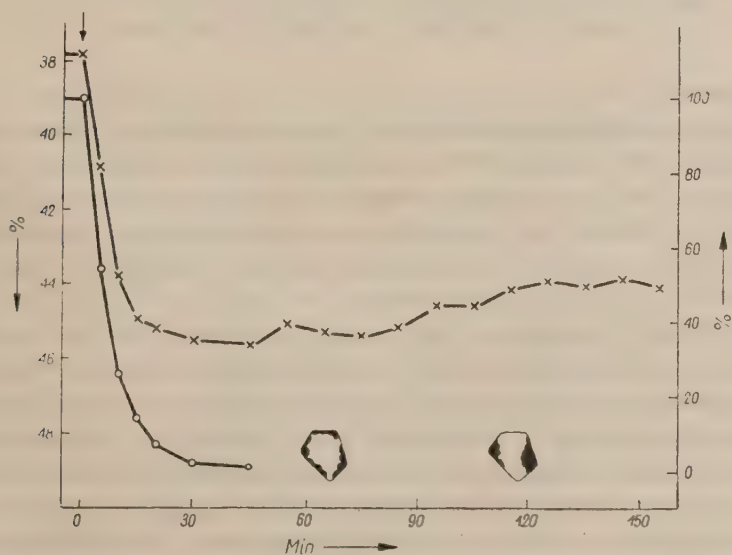


Fig. 8. *Lemna trisulca*. Changes of chloroplast arrangements and of quasi transluance recorded in blue light. Light intensity 100 kluxes. The degree of the aggregation of chloroplasts is marked schematically

decrease after long exposure to strong light experiments were carried out with *Lemna trisulca* in light of about 30 000 luxes (Fig. 7). During 7 hours of irradiation only small directionless changes of quasi translu-
tance not exceeding 2 per cent were observed. However, the results

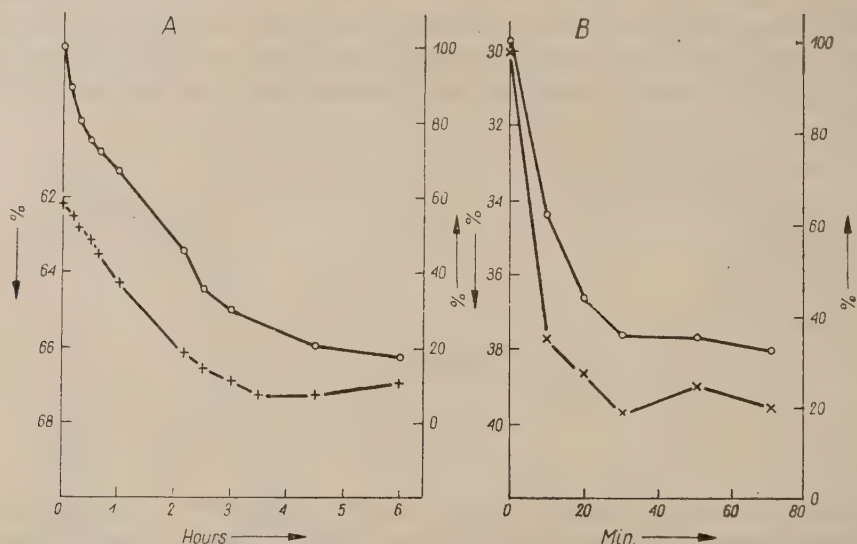


Fig. 9. A — *Funaria hygrometrica*. Changes of the arrangement of chloroplast and of quasi translu-
tance recorded in blue light. Light intensity 50 kluxes. B — *Elodea canadensis*. Changes of the arrangement of chloroplasts and of quasi translu-
tance recorded in blue light. Light intensity 30 kluxes

were different when the light intensity was about 100 000 luxes which caused the aggregation of chloroplasts. Fig. 8 representing the aggregation of chloroplasts by schematic diagrams shows that in the course of the aggregation process the tendency towards a decrease of transmission was very distinct. Since the aggregation of chloroplasts also occurs in terrestrial plants it may be an additional factor influencing the changes of the overall transmission of leaves.

On the whole analogical effects are to be found also in other water plants with assimilating mesophyll consisting of a single or a few layers. Fig. 9 shows by way of examples the changes of quasi translu-
tance in relation to the changes of chloroplast arrangements in *Funaria hygro-
metrica* and *Elodea canadensis*. In contrast to these species the leaves of *Fontinalis antipyretica* having no phototactic movements manifested an almost constant quasi translu-
tance that changed by not more than 2 per cent in spite of prolonged exposure to strong light (Fig. 10).

The results here reported indicate that quasi translu-
tance measured by the simple method of placing a photovoltaic cell in the plane of the

microscope image must be regarded as only an approximate measure of the chloroplast arrangements. The difficulties of applying this method for the determination of chloroplast arrangement consist in the high variability of quasi transluance for the two main chloroplast arrangements

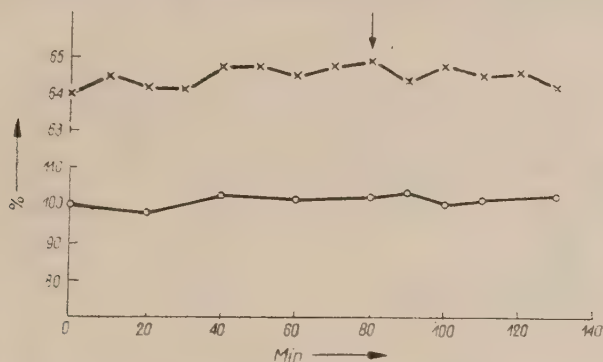


Fig. 10. *Fonitinalis antipiretica*. Changes in the number of chloroplasts in the flat arrangement and of quasi transluance in blue light. The difference in the number of chloroplasts are caused by errors in the counts

in different species and even in different plants of the same species, the absence of strict parallelism between quasi transluance and the changes in the chloroplast arrangements, and the additional disturbances arising in the case of unusual chloroplast arrangements (aggregation).

II

More accurate measurements of the optical properties of leaves with chloroplasts in the flat or profile arrangement were carried out on *Lemna trisulca*. The leaves of this plant consisting near the tip of single layer mesophyll are built at the centre of multi-layer assimilating tissue with numerous air spaces between the cells. Thus, the tip part of these leaves is very typical for the thinnest leaves (of *Funaria* type), whereas the centre part resembles more the leaves of terrestrial plants. Fig. 11 illustrates the results of measurements of semi integral transluance made at three points of a leaf: A in the single-layer part of a very young leaf, B in the single-layer part of a fully grown leaf, and C in the multi-layer part of a medium leaf. The lower transmission at point B as compared to A seemed to be due to the greater amount of the pigment (darker chloroplasts), and the lower transmission at point C as compared to A was caused by greater reflection owing to the presence of many intercellular spaces. However, in all instances semi integral transluance was distinctly lower for the profile than for the flat arrangement. The greatest

differences occurred in the short-wave part of the spectrum and in red light, i.e. for the range of strong absorption, whereas in the 500 to 600 $m\mu$ wave-length range the differences were insignificant.

The full characteristic of the changes in the optical properties of leaves taking place after the regroupment of chloroplasts is shown in

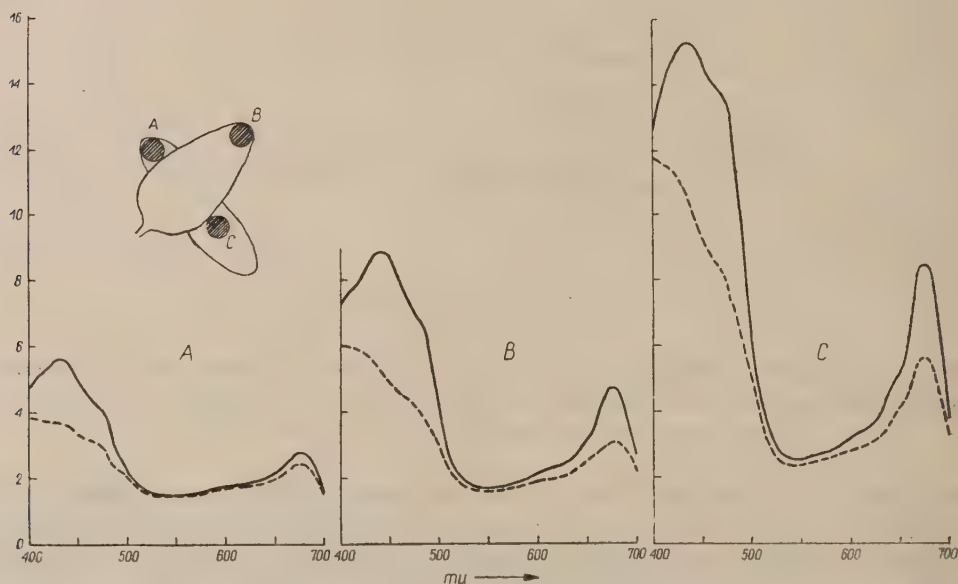


Fig. 11. *Lemna trisulca*. The dependence of semi integral transmittance on the wave length of light and the arrangement of chloroplasts for three segments of *Lemna trisulca* fronds: A — single-layer part of a young leaf, B — single-layer part of fully grown leaf, C — multi-layer part of leaf. Y-axis — reciprocal of semi integral transmittance, X-axis — wave-length

fig. 12 and 14. In the single-layer part of a leaf (fig. 12) reflection R_s was relatively small and amounted to about 3 per cent in the strong absorption range and to about 8 per cent in the middle of the spectrum. After the displacement of chloroplasts to the side cell walls reflection distinctly increased — to about 10 per cent — only in the middle part of the spectrum, whereas in the wave-length range of strong absorption the increase of reflection was insignificant (by about 0.5 per cent). Integral transmittance T_s increased very distinctly after the rearrangement of chloroplasts into the profile position, i.e. from 14 to 27 per cent in blue and from 27 to 39 per cent in red light. The differences of integral transmittance decreased with the decrease of absorption by pigments, so that at wave-lengths of about 550 $m\mu$ integral transmittance could even be somewhat lower in the profile than in the flat arrangement of chloroplast. This effect was associated distinctly with the changes of reflection

in this wave-length range. The changes of absorptance accounting for the changes of reflection and light transmission indicate a marked drop of absorption in the profile arrangement for the whole range of the spectrum, though the drop was most pronounced in the most strongly absorbed wave-length range: the drop amounted to 15 per cent in the

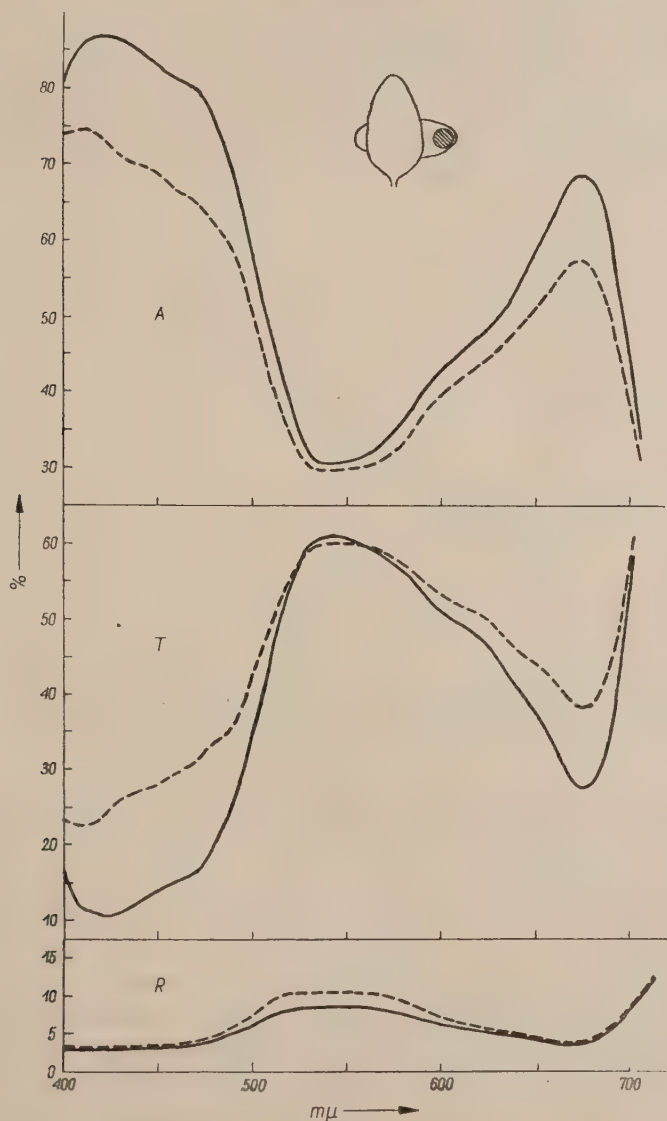


Fig. 12. Reflection (R), transmission (T) and absorption (A) spectra of the single-layer part of a *Lemna trisulca* leaf for the flat (—) and profile (---) chloroplast arrangements. X-axis — wave length; Y-axis — R; T and A in percentage

short-wave part of the spectrum, to 12 per cent in red light, and only to 2 per cent in the middle of the spectrum. The decrease of absorption in the profile arrangement was almost directly proportional to the initial absorption for epistrophe (Fig. 13), the only exception being the change of absorption in the 400 to 420 m μ wave-range which was much smaller than what could have to be expected from the initial absorption.

The results of analogical measurements of the changing optical properties of a leaf in the multi-layer part is shown in fig. 14. As compared to the thinner single-layer leaves reflection was here much higher, amounting to 19 per cent in the middle part of the spectrum, whereas integral translucance was lower. However, it is worth noting that the total amount of absorbed light energy differed only slightly in both

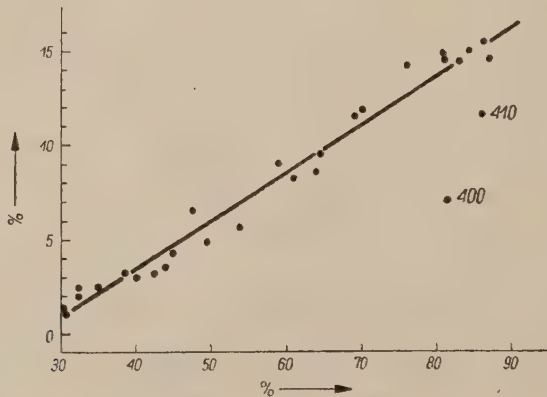


Fig. 13. The change of absorption in per cent (Y-axis) plotted against the magnitude of absorption (X-axis) for the flat arrangement of chloroplasts. The data are the same as in fig. 12. The figures alongside of some points indicate the wave-length

instances. In the case of the multi-layer part of the leaf the decrease of absorption after the chloroplasts had moved to the side walls was somewhat smaller and uniform for the whole range of the spectrum. It amounted to 10, 8 and 4 per cent respectively in blue, red and green light.

In an attempt to define more accurately the relation between the true changes of absorption and the changes of quasi translucance examined in part I of the experiments the following three values of translucance accounting for transmission and reflection in a conical beam of light of various angles were measured: rectilinear translucance in a beam of light parting at 7 degrees and containing almost exclusively parallel rays of light I_p , quasi translucance for an optical system catching rays with the maximum divergence angle of 25 degrees, and integral trans-

luctance for an opal plate. The results of these measurements carried out on the same surface of a single-layer leaf are shown in fig. 15. It is to be seen from this figure that, besides the different results of the absolute measurements of transmittance, the spectral course of the curves varies somewhat in all three instances. These differences indicate that the

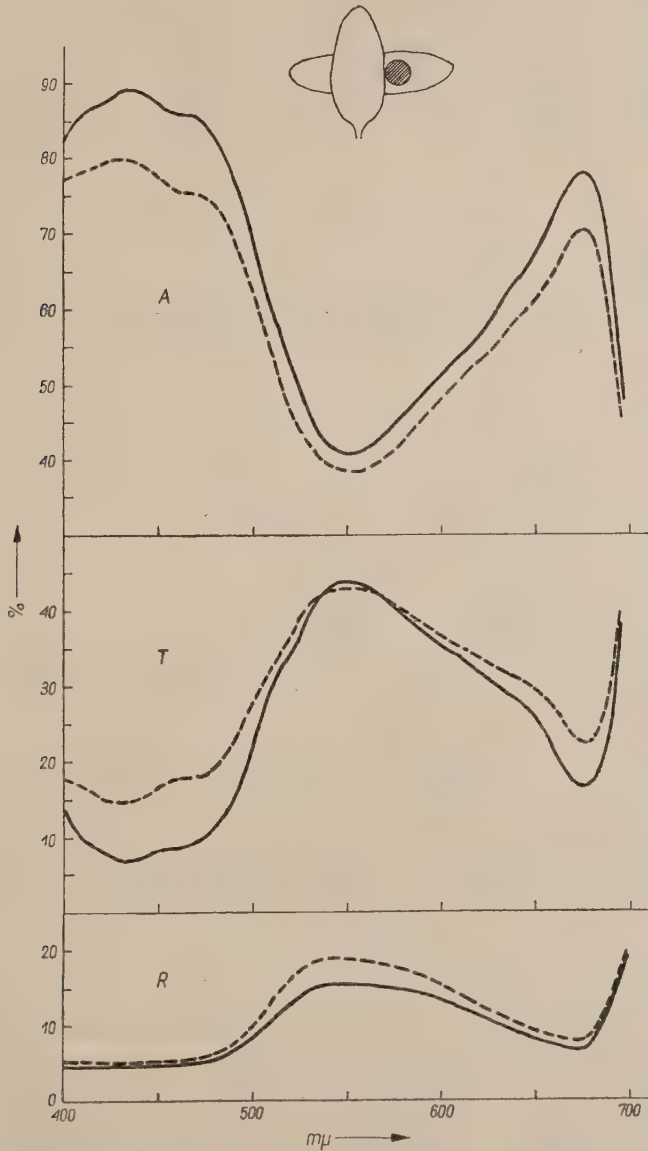


Fig. 14. Reflection, transmission and absorption spectra for the multi-layer part of a *Lemna trisulca* leaf. Details as in fig. 12

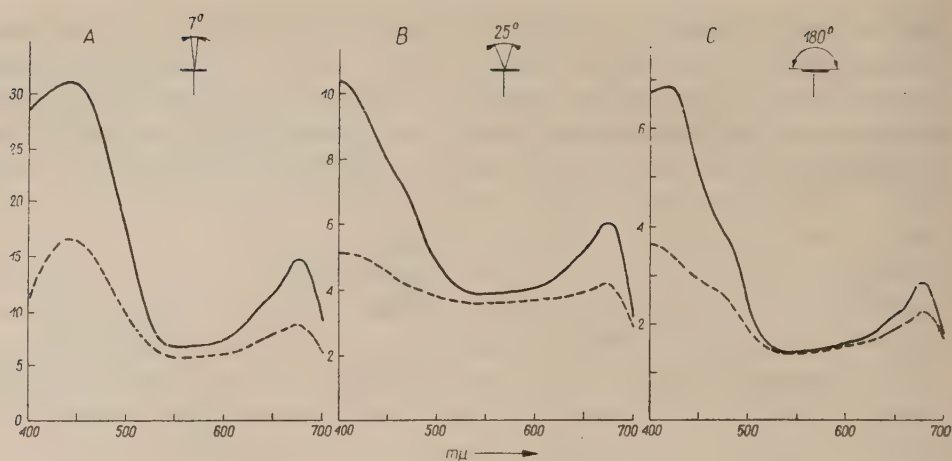


Fig. 15. Rectilinear transmittance (A), quasi transmittance (B) and semi integral transmittance (C) spectra of one segment of a leaf. X-axis — wave-length, Y-axis — reciprocal of the value of transmittance

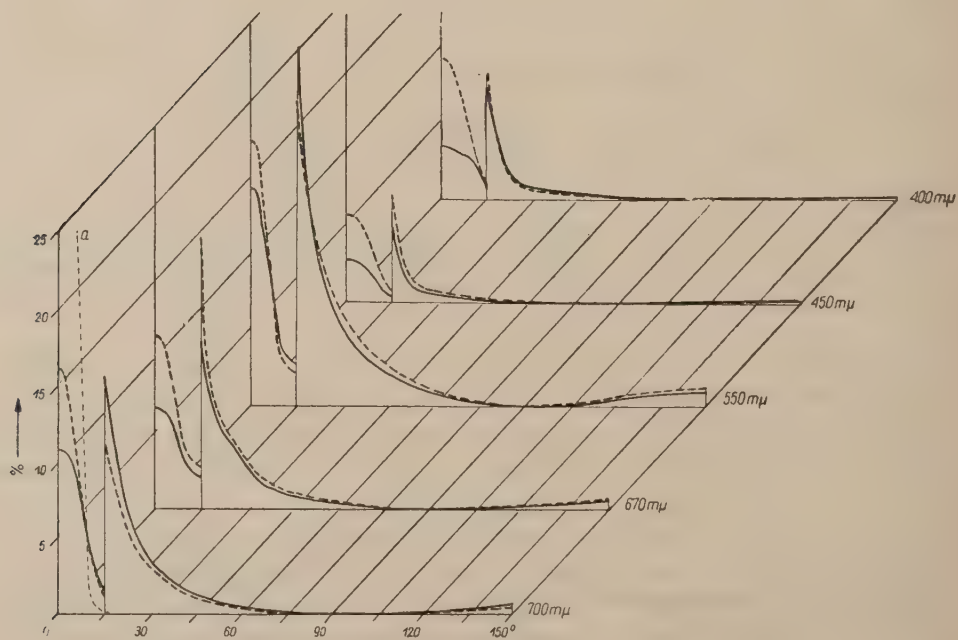


Fig. 16. Angular dispersion and reflection of the single-layer part of a *Lemna tri-sulca* leaf. X-axis — dispersion angle, Y-axis — ratio of the intensity of diffuse light to the maximum intensity of undiffuse light (from 15 degrees Y-scale 10 times enlarged)

angular dispersion of light must have been different for various wave-lengths.

The results of recordings of angular dispersion for various wave-lengths carried out on the surface of another leaf are shown in fig. 16. As is to be seen from the curves the dispersion being associated with the wave-length was small in the short-wave range and on the whole increased with increasing wave-length. Light of wave-lengths little absorbed by the leaf (550, 700 $m\mu$) was very strongly dispersed. The displacement of chloroplasts to the profile position affected above all the transmission of the undiffuse rays. The influence of a change in the

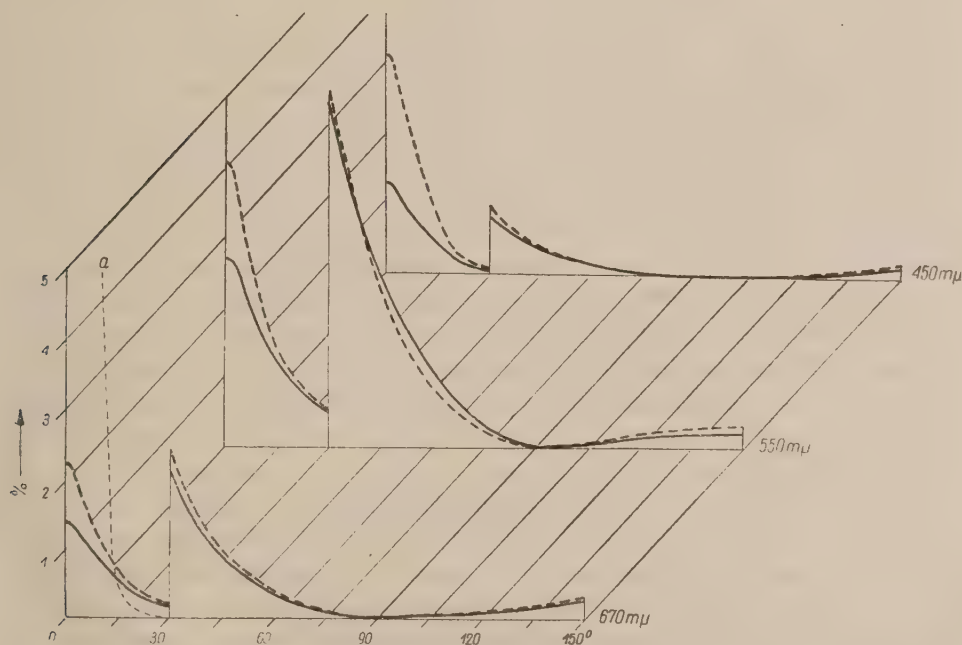


Fig. 17. Angular dispersion and reflection of the multi-layer part of a *Lemna trisulca* leaf. X-axis — dispersion angle, Y-axis — intensity of diffuse light (from 30 degrees X-scale 10 times enlarged). The curves are plotted for wave length 450, 550 and 670 $m\mu$

arrangement of chloroplasts on dispersion was rather small in the single-layer leaves. Parastrophe usually caused a somewhat greater dispersion than the flat arrangement, with the exception of the wave-length 700 $m\mu$ and to some extent 550 $m\mu$ where these relations were reversed.

The results of analogical measurements carried out on a multi-layer leaf with intercellular spaces are shown in fig. 17. The conditions with regard to translucence and reflectance of this leaf are shown in fig. 14. Owing to the different anatomical structure of the leaf transmission was

much weaker and light dispersion much stronger than in a single-layer leaf. However, the conditions in both kinds of leaves were basically similar. Dispersion increased with the increase of wave length and was especially high in light most weakly absorbed by a leaf. The change of the arrangement of chloroplasts affected most strongly the transmission of undispersed rays. The profile arrangement of chloroplasts increased dispersion, which was small in blue light, stronger in red light, and increased in light of wave length 550 m μ within the angle of 40 degrees but dropped when the divergence of rays was more than 40 degrees.

DISCUSSION

The results of the first part of experiments indicate that transmission measured microscopically as the value of quasi translucance can only be an approximate criterion of the chloroplast arrangements. The conclusion arising from the data assembled in the second part of the experiments is that the spectral sensitivity of light detector very strongly influences the experimental results. Although, the optical characteristic of leaves of terrestrial plants is much more complicated owing to the great influence of the water content of these leaves, nevertheless some of the data obtained for terrestrial plants may be interpreted on the ground of the results of this investigation. For instance, the effect observed by Biebl (1955) consisting in the increase of transmission for light photochemically active on sensitized paper and the simultaneous lack of changes in transmission when measured with a photoelement may be associated with the different spectral sensitivity of these two light detectors. Photographic paper may be sensitive to short waves (violet-blue light) and in this wave-length range the changes of transmission are in fact appreciable. On the other hand the photoelement may be mainly sensitive to yellow-green light and in this range the changes of transmission are much smaller. In view of these results Babuskin's (1955) method of determining the arrangement of chloroplasts from the density of exposed sensitized paper seems to be of insufficient accuracy for investigations on the action spectrum.

In all the measurements of this investigation a decrease of light absorption has been observed when the chloroplasts passed from the flat to the profile arrangement. In single-layer leaves this decrease is very well marked in strongly absorbed light and much less apparent in yellow-green light. In multi-layer leaves the differences of light absorption are only in a small degree dependent on the spectral composition of light. The data obtained for this last kind of leaves approach more the results reported by Seybold (1956) for terrestrial plants; this worker obser-

ved a decrease of absorption by about 3 to 5 per cent more or less uniform over the whole spectrum. The differences of absorption between the flat and the profile arrangement of chloroplasts are with satisfactory approximation directly proportional to the total absorption within a given part of the spectrum. The decrease of absorption is distinctly smaller only in the short-wave end of the spectrum (below 420 m μ). A similar effect, though less distinctly marked, is also visible on the curves plotted by Seybold (1956) and is probably associated with the rising absorption by other elements of a cell in the short wave-length range. Metzner (1930) demonstrated the weak absorption of long wave ultraviolet radiation by the cytoplasm and nuclei and the much greater absorption by the cell sap of the epidermis and mesophyll of many plants. The observations of Seybold and Weissweiler (1942) show that the leaves of *Pelargonium zonale* containing assimilating pigments absorb about 20 per cent of medium and long wave light, whereas at 400 m μ their absorption increases to about 50 per cent. For almost the whole range of the spectrum the photosynthetic pigments have the greatest influence on absorption and thus the rearrangements of chloroplasts are associated with the changes of absorption proportional to the light absorbed in these pigments. In the short wave range absorption of light in other parts of a cell which are not rearranged tends to play a greater role and this seems to be the explanation of the relatively small influence of chloroplast displacements on the changes of absorption.

Table 2

Decrease of the absorption of light when
chloroplasts pass to the profile arrange-
ment

(Zurzycki 1953, mean values from table 4-
Absorption in the flat arrangement = 100%)

Log E of a chloro- plast	Decrease of absorption %
3.5	1.79
4	6.52
4.5	16.5
5	33.4

This author (Zurzycki 1953) has theoretically calculated the changes of light absorption from the decrease of the surface area of chloroplasts directly exposed to light and the simultaneous increase of the thickness of the absorbing layer. In the case of *Lemna trisulca* the decrease of the surface exposed to light amounts to 50—60 per cent. The

Table 3

Decrease of the absorption of light after chloroplasts
passed to the profile arrangement
(Absorption in the flat arrangement = 100%)

Wave lenght m μ	Decrease of absorption in parastrophe %
440	18.8
480	18.4
550	4.5
600	7.5
670	17.8

calculated changes of light absorption for some given extinction of a chloroplast are assembled in table 2. The value of log E for a chloroplast ranges 3.5 to 4.4. The changes of absorption obtained experimentally and shown in fig. 12 are listed in table 3. As is to be seen the data in these two tables are in very good agreement except for the middle part of the spectrum where the changes of absorption are mainly due to reflection, which factor has not been included in the theoretical calculations. This agreement between the theoretical and experimental values of light absorption at least so far as the order of the magnitudes involved is concerned, proves that the theoretical principles underlying the calculations (Zurzycki 1953) are correct. One of these assumptions has been the uniform distribution of light throughout the whole crosssection of the cell. It seems, therefore, that the refraction of light at the edges of a cell has much less significance than has been believed hitherto (Senn 1908, Zurzycki, 1953, also Haupt 1959). When comparing the results of this investigation with the influence of the displacement of chloroplasts on the rate of photosynthesis (Zurzycki 1955) it is to be seen that, though the decrease of absorption in the profile with regard to the flat arrangements is only 12 per cent, the effect of the chloroplast displacement on photosynthesis is much greater. Some experimental data illustrating this point are assembled in table 4.

The differences in the rate of photosynthesis are of the order of 50 per cent and they thus exceed several times the changes of light absorption. The question now arising is whether this discrepancy is caused by the additional influence exerted by the induction period of photosynthesis or by the different photosynthetic capacity with regard to the amount of absorbed energy of chloroplasts in the profile than in the flat arrangement. The latter supposition may be supported by the

Table 4

Changes of the rate of photosynthesis after chloroplasts passed to the profile arrangement (photosynthesis for the flat arrangement epistrophe = 100%)

No. of experiment	Respiration $10^{-3} \mu\text{l}/10 \text{ min}$	True photosynthesis		% reduction of photosynthesis rate
		epistrophe	parastrophe	
L 8	— 5.63	0.82	1.61	49.0
L11	—11.21	5.50	9.61	42.8
L20	—14.10	8.23	17.60	53.3

lamellar structure of chloroplasts and the partial orientation of the pigment molecules in this structure (Goedheer 1957).

The angular dispersion in leaves of *Lemna trisulca* is much smaller than in the leaves of terrestrial plants (Seybold 1933, Metzner 1957). Here again the multi-layer parts of the *Lemna* leaves occupy the intermediate position between the very weakly dispersing leaves having a single-layer mesophyll tissue and the leaves of terrestrial plants. According to Pokrowski (1925) light reflected from a leaf consists partly of light reflected from the external surface and partly of light reflected from the inner surfaces of the leaf. This latter component consists of light more strongly dispersed in various directions. The comparison of the light reflection and dispersion of single-layer *Lemna* leaves, of multi-layer *Lemna* leaves and of the leaves of terrestrial plants shows how relative amount of diffuse light increases owing to the growing complexity of the anatomic structure and of the intercellular system.

In the measurements of angular dispersion no maximum has been recorded, though the existence of such a maximum has seemed probable in connection with the interference of light in the chloroplasts when they are in the profile arrangement (Menke 1957). The interference effect is either very weak or very indistinct owing to the differences in the positions of the particular chloroplasts.

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SUMMARY

1. A simple microscopic apparatus provided with a photovoltaic cell has made possible the demonstration of a certain parallelism between the changes in the quasi transmittance of a leaf and the rearrangement of chloroplasts. However, the changes of quasi transmittance cannot be taken as an accurate criterion of the chloroplast arrangements.

2. The changes in the true absorption of leaves when the chloroplasts pass from the flat to the profile arrangement depend on the total absorption; in *Lemna trisulca* leaves with a single-layer mesophyll they range from about 1 per cent in green light to about 12 per cent in red light and 15 per cent in blue light. The corresponding relative changes expressed in percentages of the absorption in leaves with a flat arrangement of chloroplasts are 19, 18 and 4.5 for blue, red and green light respectively. The average decrease of absorption for the profile arrangement as compared to the flat arrangement may be accepted to be 10 to 12 per cent for the wave-length range 400 to 700 m μ .

3. The leaves of *Lemna trisulca* with a multi-layer mesophyll manifest a lower transmittance, greater reflection and greater dispersion than the leaves with a single-layer mesophyll. In the multi-layer leaves the changes of absorption when the chloroplasts pass to the profile arrangement are not so strongly dependent upon the wave length as in leaves with a single layer.

4. The comparison of the experimental results with the theoretically calculated changes of absorption and with the measurements of the rate of photosynthesis point to the small significance at any rate in leaves with a single-layer mesophyll, of the local reduction of light intensity on the side cell walls. The reduction of the rate of photosynthesis when the chloroplasts are in the profile arrangement seems to be greater than could be expected from the changes in the absorption of light energy.

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Migration of nuclei in tetrapolar *Basidiomycetes*

K. M. ŚWIEŻYŃSKI

When two compatible monokaryotic strains meet, a cytoplasmic connection is established between their hyphae and each mycelium is invaded by nuclei from the other strain. In this way both strains are transformed into dikaryotic mycelia. This invasion of nuclei was first analysed by Buller (1931) in *Coprinus* and was later shown to exist in many species and genera of both *Basidiomycetes* and *Ascomycetes* (Burnett 1956, Snider and Raper 1958, Świeżyński and Day 1960b). The phenomenon is called migration of nuclei.

In tetrapolar *Basidiomycetes* the mating type is determined by two independently inherited loci: *A* and *B*, each with multiple alleles. Only strains with different alleles at both *A* and *B* loci are compatible. Most of the detailed information concerning migration of nuclei comes from research on tetrapolar *Basidiomycetes*. As it appears unlikely that the migration of nuclei has the same mechanism in all groups of fungi in which it has been demonstrated, the discussion in this paper will be limited to tetrapolar *Basidiomycetes*.

The basic facts concerning migration of nuclei in tetrapolar *Basidiomycetes* may be summarised as follows:

1. Hyphae establish cytoplasmic connections more or less independently of mating type. This is shown by the existence in various fungi of common *A*, common *B* and common *AB* heterokaryons (review: Papazian 1958).

2. The *A* and *B* mating type factors are not functionally alike. The difference in *B* alleles appears to be decisive for migration, and difference in *A* alleles — for conjugated divisions of nuclei (Świeżyński and Day 1960a).

3. If nuclei of paired monokaryotic mycelia have different *B* alleles, migration of nuclei is observed (Świeżyński and Day 1960b). As Papazian (1958) points out: "the most acceptable interpretation is that invading nuclei migrate through the cells of a compatible mycelium". The alternative hypothesis, that invading hyphae grow throughout the compatible mycelium is difficult to accept for various reasons:

- a) The rate of migration of nuclei is much higher than the rate of growth of hyphae (Snider and Raper 1958).

b) Migrant nuclei do not colonise densely the invaded mycelium. Migrants may be found in places surrounded by areas in which their presence cannot be detected (Snider and Raper 1958).

c) Mutual inhibition of strains is not directly connected with mating type. It is difficult therefore to accept that mating type has a decisive influence on the intergrowth of hyphae.

For all these reasons it is accepted that nuclei migrate through the cells of the invaded mycelium.

4. Compatible nuclei migrate only if there are no limitations of migration. Primary limitations of migration (Brodie 1948) are found in cases in which a monokaryon is unable to accept foreign nuclei, whether compatible or not. Secondary limitation of migration is observed in mycelia in which migration of nuclei occurs, but is not complete and single hyphae with one type of nuclei may be isolated from heterokaryotised areas (Świeżyński and Day 1960b).

5. Dikaryotic and heterokaryotic mycelia with common *B* factor donate but won't accept nuclei (Świeżyński and Day 1960a).

To the author's knowledge, no general working hypothesis has been published which would explain the phenomena observed in migration of nuclei. An attempt will be made in this paper to formulate such an hypothesis.

It appears, that facts observed in migration of nuclei in tetrapolar *Basidiomycetes* may be explained if the following model of the action of the incompatibility factors is accepted:

1. A single nucleus induces round it a "field" in which all nuclei possessing different *B* alleles are attracted to this nucleus.

2. In conjugated nuclei (situation occurring in dikaryons and common *B* heterokaryons) the forces inducing migration are reduced or are not present. Therefore conjugated nuclei will not attract foreign nuclei. On the other hand conjugated nuclei are liable to attraction by single nuclei. Single nuclei may attract one only or both the conjugated nuclei. The inability to attract foreign nuclei by conjugated ones may be due to the presence of 2 different *A* factors in direct vicinity or to the presence of 2 neighbouring *B* factors (different or alike).

3. There exist factors which limit or completely suppress the "field" exerted by the nucleus. The disappearance of the "field" results in primary and secondary limitation of migration.

4. There exist factors influencing the extent of response of the migrant nucleus to the "field", i.e. factors influencing the rate of migration. Here may operate differences in alleles of the *A* and *B* loci (for instance nuclei with different *A* alleles may be attracted with different force) and other modifying agents, heritable or not.

Summing up, the proposed hypothesis is based on two assumptions: first, that nuclei will migrate only if a force attracting them is present, and second, that the attraction is directed, i.e. if one nucleus is attracted by the other, the reverse may not be true.

If the above model is accepted, it becomes possible to explain several facts observed in experimental work.

THE MIGRATION OF NUCLEI IN COMPATIBLE MONOKARYOTIC MYCELIA

It is explained as follows: After establishment of cytoplasmic connections between hyphae the nuclei will migrate into the foreign mycelium because they are attracted by the nearest single nuclei. If the reaction is not unilateral, it may depend on chance, nutrition (Holton 1942) or other factors which hypha will be invaded in each particular case. As soon as the first dikaryon is formed the mutual attracting forces will dwindle and the migrant nucleus will be attracted by the next single nucleus. In this way the nuclei will migrate in the mycelium of the host. Basing on this model we may understand the observation of Snider and Raper 1958, that migrant nuclei do not colonise densely the host mycelium and there are areas passed by the migrant nuclei in which they cannot be detected.

MIGRATION OF SINGLE NUCLEI IN OTHER COMBINATIONS OF MYCELIA

In several *Basidiomycetes* it could be demonstrated that dikaryons may act as donors of nuclei. The fact was first demonstrated in *Coprinus lagopus* by Buller (1930) and then shown to exist in many other *Basidiomycetes* (Kimura 1958). On the other hand migration of nuclei has never been observed in dikaryotic mycelia (Świeżyński and Day 1960b). These facts are explained simply if the thesis 2 of the proposed model is accepted stating that conjugated nuclei are unable to attract foreign nuclei, but may be attracted by single nuclei.

The common *B* heterokaryons act as donors but not as acceptors of nuclei in the same way as dikaryons do it (Świeżyński and Day 1960a) and the explanation of the facts seems to be similar.

Common *A* and common *AB* heterokaryons will usually act as donors and as acceptors of nuclei (Świeżyński and Day 1960a). Such heterokaryons are much less stable than dikaryons and common *B* heterokaryons and frequently produce hyphae with only one type of nucleus (Raper and San Antonio 1954, Świeżyński and Day 1960a), what may account for the fact that they usually behave like monokaryons as far as migration of nuclei is concerned.

MIGRATION OF BOTH NUCLEI IN COMPATIBLE AND DOUBLY COMPATIBLE DI — MON MATINGS

It has been shown that if a small dikaryotic inoculum is added to a large monokaryotic mycelium, occasionally both nuclei of the added dikaryon migrate through the monokaryotic mycelium and establish on its margin the original dikaryon. It may happen in doubly compatible i.e. $(A_1B_1 \times A_2B_2) \times A_3B_3$, compatible, i.e. $(A_1B_1 \times A_2B_2) \times A_2B_2$ and incompatible, i.e. $(A_1B_1 \times A_2B_2) \times A_1B_2$ matings.

Migration of both nuclei in an incompatible mating has been explained in a simple way elsewhere (Świeżyński and Day 1960b). In *Coprinus fimetarius*, *C. macrorrhizus*, *Galera tenera* and *Schizophyllum commune* it has been demonstrated (Quintanilha 1939, Oikawa 1939, Papazian 1950, Crowe 1958, Kimura 1958) that both nuclei may migrate in compatible and doubly compatible matings. The explanation of migration of both nuclei in doubly compatible matings may be fairly simple. It is enough to assume that by chance some hyphae are invaded by one type and other hyphae by the other type of nucleus of the dikaryon. Oikawa demonstrated that this may happen. In a doubly compatible cross he observed that one part of the monokaryotic strain was dikaryotised by one and another part by the other nucleus of the original dikaryon. As dikaryons may exchange nuclei to form recombinant dikaryons (Świeżyński 1961) it may be inferred that migration of both nuclei results from individual migration of single nuclei and then formation of a recombinant dikaryon. This reasoning will not explain the migration of both nuclei in compatible crosses of the type: $(A_1B_1 \times A_2B_2) \times A_3B_2$. Kimura (1958) demonstrated that also in such combinations both nuclei may migrate. It appears that this fact may be understood only by assuming that both conjugated nuclei of the dikaryon migrate as one unit, being jointly attracted by single nuclei with a *B* allele common to one of the nuclei of the dikaryon. It is possible that this happens if a diploid nucleus is formed. The existence of such nuclei is not improbable as recombinant nuclei have also been observed in such matings (Papazian 1950, Crowe 1958, Kimura 1958). This alternative explanation may apply also to migration of both nuclei in doubly compatible matings.

LIMITATIONS AND VARIATIONS IN RATE OF MIGRATION

Fulton (1950) suggested that in *Cyathus stercoreus* limitations of migration may be connected with the existence of loose pairing of nuclei, which appears to be due not to the factors determining mating type but to some other factors. Mycelia with such associations of nuclei acted as acceptors and not as donors of nuclei.

Various authors observed dedikaryotisation and secondary limitations of migration (Świeżyński 1961, reviews: Buller 1941, Raper 1953). This indicates that limitations of migration may evolve during the development of dikaryotic and heterokaryotic mycelia. The nature of these limitations is not yet clear.

Quintanilha (1938, 1939), Papazian (1950) and Kimura (1958) demonstrated that in doubly compatible matings the nuclei do not migrate at random, but that definite combinations of nuclei are prevalent. The results of Papazian cannot be explained on the assumption that only differences in *B* alleles are responsible for this fact. The results of Quintanilha and Kimura indicate that some modifiers, inherited independently from the mating type loci, are in operation.

All these data show that mating type loci are not the only factors influencing the migration of nuclei. The existence of some modifying factors must be assumed as well, but their nature still remains obscure.

*

The proposed model is based mainly on genetical evidence due to the fact that cytological and biochemical evidence is still very scarce. The studies of Snider and Raper (1958) indicate that the migration is due to chemical rather than physical processes. In formulating the model the idea of Quintanilha (1939) that different mating factors may attract each other has been accepted as well as the idea of Lewis (1954) that these forces may be neutralised in conjugated nuclei. There is no need to accept any kind of repulsion between mating type loci to explain phenomena of migration and it appears still to be an open question whether differences in the rate of migration may be ascribed to differences in *B* alleles or are due exclusively to other factors.

The proposed model may be proved only if a material basis for the attraction of nuclei may be demonstrated and if it will function in the predicted way. On the other hand the model may be shown to be wrong or require alterations if contradictory facts are found.

It has been formulated in the hope that it may help to define the points which still remain obscure and establish the material basis of the facts observed.

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At the same time the author wants to state that he is solely responsible for the opinions presented in this paper.

SUMMARY

A hypothetical model of nuclear migration in tetrapolar *Basidiomycetes* is suggested. It is based on two assumptions: first, that nuclei will migrate only if a force attracting them is present, and second, that the attraction is directed, i.e. if one nucleus is attracted by the other, the reverse may not be true.

It is attempted to explain basic facts observed in migration of nuclei with the use of this model.

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Exchange of nuclei between dikaryons in *Coprinus lagopus*

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The research of Pontecorvo and his school (1959) has focussed attention on the importance of parasexual processes in fungi which bring about exchange of genetic material without the normal sexual cycle. One of the simplest ways of exchanging genetic material in dikaryotic mycelia of *Basidiomycetes* would be an exchange of nuclei between two dikaryons leading to the formation of a recombinant dikaryon.

As far as the author is aware, there is only one published example of such an exchange in *Holobasidiomycetes*. Kimura (1958) fruited jointly pairs of dikaryotic mycelia of *Coprinus macrorhizus* and found that most fruitbodies were formed by the components, but one fruitbody apparently originated from a recombinant mycelium. Several examples of exchange of nuclei in rusts were described (Ellingbøe 1961, review: Day 1960b). In this group of fungi however the dikaryotic mycelium appears to be less stable and multinucleate hyphae were observed several times (Allen 1926, Nelson a.a., 1955).

The present study was undertaken to find out, whether recombinant dikaryons may be formed in *Coprinus lagopus*.

MATERIALS AND METHODS

Coprinus strains, used in this study, come from wild-type and mutant strains kindly supplied by Dr. P. R. Day from the John Innes Institute, England. Their origin is described in previous papers (Świeżyński and Day 1960a,b).

Three alleles of the *A* mating type locus i.e. A_2 , A_5 and A_6 , three alleles of the *B* mating type locus i.e. B_1 , B_5 and B_6 , and three auxotrophic mutants. $a = ad$ 8, $m = me$ 5 and $c = chol$ 1 with nutrient requirement of adenine, methionine and choline respectively, were used in this study. These loci fall into two linkage groups. The loci a and m are linked with A , the distance $A - a$ being 1.27 units and $A - m$ being 23 units. In the second linkage group B is linked with c at a distance calculated from different crosses to be 5.94 to 15.4 units (Day 1958, 1960a).

Recombinants were obtained from the above material by making single basidiospore isolations from suitable matings.

All the experiments, unless otherwise stated, were performed at 30° C in Petri dishes 80 mm. in diameter. Three types of nutrient media were used:

a) A simplified Fries (1953) minimal medium. In 1 litre it contains 20 g. glucose, 2 g. asparagine, 0.5 g. ammonium tartrate, 1 g. KH_2PO_4 , 2.25 g. Na_2HPO_4 , 0.29 g. Na_2SO_4 , 0.04 mg thiamin and 20 g. agar.

b) Minimal medium supplemented with adenine (0.01 g/litre), methionine (0.01 g/litre) or choline (0.2 mg/litre), singly or doubly, and termed respectively: a, m, c, am, ac and mc. For instance mc is a minimal medium supplemented with methionine and choline.

c) A modified Day (1959) complete medium. It contains all the components of the minimal medium and moreover to each litre are added: 1.35 g. yeast extract, 0.75 g. peptone and 1.25 ml. hydrolysed yeast nucleic acid.

Fruitbodies were produced in bottles of 500 ml volume on sterilised horse manure at about 26° C.

To test the possibility of exchange of nuclei between dikaryons, blocks of 1 mm. side of 2 different dikaryotic mycelia were placed on Petri dishes side by side and allowed to grow jointly. Usually 3 inocula (or pairs of inocula) were placed in the centre of the Petri dish (in an equilateral triangle, 15 mm. side). The extent of growth was established by measuring the largest distance from the inoculum to the margin of the colony (the radius). In each dikaryon both component nuclei were double mutants and had one nutrient requirement in common. The paired dikaryons had one nucleus in common, but each dikaryon of the pair was homozygous for a different nutrient requirement. The 3 dissimilar nuclei present in each pair of dikaryons were mutually compatible. To detect any influence of cytoplasm dikaryons were synthesised in both directions. Reciprocal matings were not always successful, because some strains were unable to become dikaryotised. This limited the variety of combinations. All the investigated combinations of dikaryons are presented in table 1. For instance in the combination of type I^a the component nuclei of the first dikaryon require methionine and choline (one nucleus) and adenine and methionine (the other nucleus). Both nuclei require methionine and therefore the mycelium with the first dikaryon will grow only on minimal medium supplemented with methionine. In a similar way the second dikaryotic mycelium will grow only on minimal medium supplemented with adenine. A recombinant dikaryon may be recognised if it is composed of the nucleus A_2B_1 mc from the first dikaryon, and the nucleus A_5B_5 ac from the second one. A mycelium with such a recombinant

Table 1
Investigated combinations of dikaryons

Type	Component dikaryons	Nutrient requirements of the dikaryons		
		first	second	recombinat
I	a	m*	a**	c***
	b			
II	a	a	c	m
	b			
III	a	c	m	a
	b			

Notice: Strain used as acceptor of nuclei is underlined

* methionine

** adenine

*** choline

dikaryon would require choline. Only cytoplasmic differences exist between type I^a and I^b. Types I, II and III differ in nutrient requirements of the component dikaryons and in mating types. Combinations of *A* and *B* alleles with auxotrophic mutants could not be chosen freely as not all possible combinations were available.

Samples of mycelia or single basidiospore isolates were tested for nutrient requirement and mating type as follows:

To test nutrient requirement, unless otherwise stated, samples of mycelium were inoculated to 3 doubly supplemented media: am, ac and mc respectively. Prototrophic mycelia were able to grow on each of them, and auxotrophic mycelia requiring all the compounds were unable to grow on any of them. Auxotrophic mycelia with single requirements were unable to grow on one of the media, and doubly auxotrophic mycelia were unable to grow on two of them.

To determine the mating type 4 wild type tester stocks were used, each compatible for one mating type locus with all the strains. For instance if spores originating from a dikaryon: $A_5B_5 \times A_6B_6$ were analysed, the following strains were used as tester stocks: A_5B_1 , A_6B_1 , A_2B_5 and A_2B_6 .

The determination of nutrient requirement was usually made first to establish the expected mating type alleles.

PRELIMINARY EXPERIMENTS

Each of the 10 dikaryotic mycelia used in the combinations listed in table 1 was tested on minimal and supplemented media. All had the expected growth requirements. They were also allowed to fruit in dung bottles. Each bottle was inoculated with a different dikaryotic mycelium. Two of them, used twice in combinations listed on table 1 (because reciprocal combinations could not be obtained) were inoculated to two dung bottles each. All bottles with methionine requiring mycelia fruited, producing 1—4 fruitbodies in the bottle. Only 2 fruitbodies were obtained from choline requiring mycelia and one of them with great delay (after 20 days). No fruitbodies were obtained from mycelia requiring adenine (table 2). Spore analysis was made from 2 fruitbodies developed from methionine requiring mycelia and 1 fruitbody developed from a choline requiring mycelium. The spores were found to have the expected growth requirements and mating type alleles.

Each dikaryotic mycelium was also grown on complete medium in Petri dishes. Their growth was more or less normal. The margin of the colonies was even, but usually the margin of the submerged growth extended further than the margin of the surface growth. The average

Table 2
Growth, fruiting ability and hyphal tip characteristic of the component dikaryons

Dikaryon	Growth mm*	Number of		Dikaryons	Number of hyphae tip isolates			lost	total
		bottles	fruitbodies		Monokaryons with requirement of:				
					ac	am	mc		
$A_2B_1 \frac{mc}{mc} \times A_6B_6 \frac{am}{am}$	23	1	4	16	—	—	1	1	18
$A_2B_1 \frac{mc}{mc} \times A_6B_6 \frac{am}{am}$	24	1	1	18	—	—	—	—	18
$A_2B_6 \frac{mc}{mc} \times A_5B_1 \frac{am}{am}$	22	2	3	13**	—	3	1	1	18
$A_6B_6 \frac{am}{am} \times A_5B_5 \frac{ac}{ac}$	22	1	—	8	4	3	—	3	18
$A_6B_6 \frac{am}{am} \times A_5B_5 \frac{ac}{ac}$	24	1	—	13	2	1	—	2	18
$A_6B_1 \frac{am}{am} \times A_5B_6 \frac{ac}{ac}$	25	1	—	15	2	1	—	—	18
$A_6B_1 \frac{am}{am} \times A_5B_6 \frac{ac}{ac}$	27	1	—	18	—	—	—	—	18
$A_5B_6 \frac{ac}{ac} \times A_2B_5 \frac{mc}{mc}$	30	2	1	15	1	—	1	1	18
$A_6B_5 \frac{ac}{ac} \times A_2B_6 \frac{mc}{mc}$	29	1	1	16	1	—	1	—	18
$A_6B_5 \frac{ac}{ac} \times A_2B_6 \frac{mc}{mc}$	23	1	—	17	—	—	1	—	18
Total		12	10	149	10	8	5	8	180

* Average radius of the 3 colonies growing on complete medium in Petri dishes, measured after 6 days of growth.

** Ten of the dikaryons required both adenine and methionine.

radius of the colonies after 6 days of growth ranged from 22—30 mm. (table 2).

The stability of the dikaryons was checked by making hyphal tip isolates. After 5 days of growth on complete medium, 16 samples, each containing a few hyphae, were taken from the margin of the mycelium of each plate. Of these 8 samples contained only hyphal tips growing under the surface of the agar, and 8 samples contained hyphae growing both on and under the surface. The samples were transferred to complete medium. After 24 hours some hyphae easy to isolate individually grew from the inocula. From samples of each dikaryotic mycelium 18 single hyphal tips were isolated (9 from the submerged and 9 from the mixed parts). After 48 hours the isolates developed into colonies. They were first tested for ability to grow on minimal medium supplemented with the compound required by the original dikaryon. If they were unable to grow, their nutrient requirement was determined and they were checked for the presence of clamps. Out of 180 isolates made, 8 did not grow, 23 appeared to be monokaryotic and the remainder were dikaryotic. Both types of monokaryotic mycelia could be isolated sometimes from hyphal tips of a given dikaryotic mycelium. An interesting case was observed in one dikaryotic mycelium requiring methionine. Out of 13 isolates with clamps (apparently dikaryotic), 10 required both adenine and methionine for growth (table 2).

Of the 23 monokaryotic hyphal tip isolates, 15 originated from samples taken from submerged hyphae.

GROWTH OF PAIRS OF DIKARYOTIC MYCELIA ON COMPLETE MEDIUM

The six pairs of dikaryotic mycelia listed on table 1 were inoculated to plates of complete medium. Each pair was inoculated to a separate plate. The mycelia were allowed to grow for 6 days and this group of plates is called transfer 1. On the 6th day from each plate blocks of ca. 3 mm. side of dense mycelium, developing in the area where both mycelia met, were used to inoculate new plates. Again 6 plates were inoculated and grown for 6 days. This group of plates is called transfer 2. In the same way mycelia of transfer 3 were grown from inocula taken from plates of transfer 2.

After 6 days each plate was covered or nearly covered with mycelium. The transfer 1 mycelia tended to form separate sectors, between which areas of dense mycelium of various size and shape could be found (Plate I, fig. 1 and 2). In later transfers the growth was more intermixed, but the dense areas could still be found, sometimes of considerable size (Plate I, fig. 3 and 4). No such areas of dense mycelium were found when single dikaryotic mycelia were grown on complete medium.

Plate I

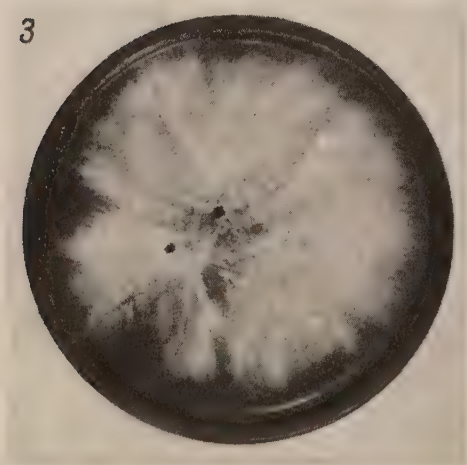
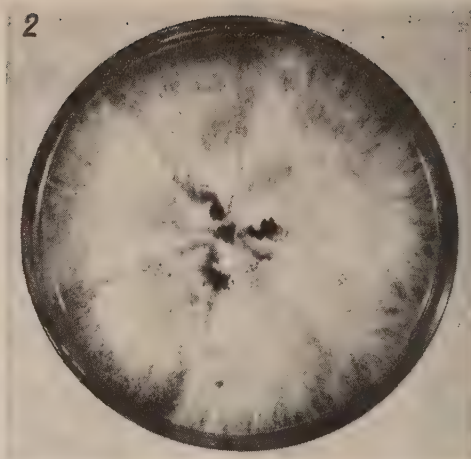
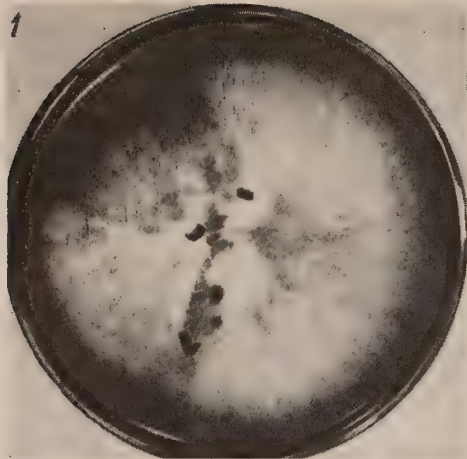




Fig. 1. — Pairs of dikaryotic mycelia inoculated to complete medium after 6 days of growth. Transfer 1, combination of type II. Different growth of both dikaryotic mycelia and dense growth in the meeting area visible



Fig. 2. — As fig. 1, but transfer 1, type I. Both dikaryotic mycelia growing vigorously, dense areas less pronounced.

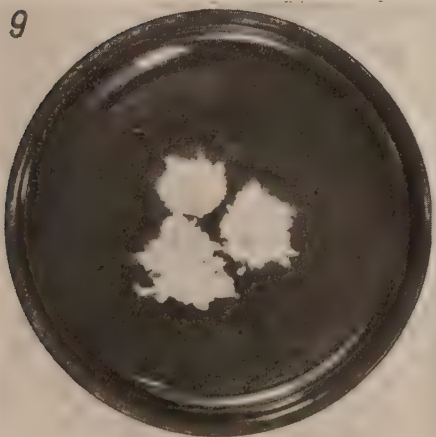


Fig. 3. — As fig. 1, but transfer 3, type I. Dense areas surround the inocula and spread in stripes

Fig. 4. — As fig. 1, but transfer 3, type II. Uneven growth of mycelia is visible. Dense areas are less pronounced

Fig. 5. — Recombinant dikaryotic mycelium auxotrophic for adenine, after 6 days of growth on minimal medium supplemented with adenine

Fig. 6. — Pairs of dikaryotic mycelia inoculated to minimal medium after 6 days of growth. Transfer 1, type I. Sectors with vigorous growth and sectors with faint growth clearly visible

Fig. 7—9. — The same series as fig. 6, but various kinds of growth in transfer 3: Fig. 7 presents mycelia in combination of type I, fig 8 — type II and fig. 9 — type III. Further explanations in the text.

Pictures reproduced in fig. 1—9 made by S. Kowalski

Table 3

Growth on minimal medium of samples isolated from complete medium

Type*		Samples from second transfer		Samples from third transfer	
		isolated	growing on minimal medium	isolated	growing on minimal medium
I	a	3	3	3	3
	b	3	3	3	1
II	a	3	2	3	2
	b	3	3	3	2
III	a	3	3	3	1
	b	3	1	3	3
Total		18	15	18	12

* Cf. table 1

After 6 days of growth, blocks of 1 mm. side were taken from dense areas of the second and the third transfer to inoculate minimal plates. The results are presented on table 3. Most isolates were able to grow on minimal medium. This indicates that in dense growing areas nuclei of both original dikaryons are usually present.

FRUITING ABILITY OF DIKARYOTIC MYCELIA GROWING JOINTLY ON COMPLETE MEDIUM

After 6 days of growth, samples of dense mycelium of each of the 3 transfers were inoculated to dung bottles, to test their fruiting ability. From each plate 1 bottle was inoculated, samples being taken from all the 3 colonies growing on each plate. The results are presented in table 4. Mycelia in each bottle fruited, frequently producing several fruitbodies. The fruiting was completed within 12 days from inoculation.

Single basidiospore isolates from nine fruitbodies (3 from each of the three types listed in table 1) were analysed, their growth requirements and mating type being determined.

Abnormalities were found in 1 fruitbody of type I and 1 fruitbody of type III. Therefore from these two types new samples, 5 fruit bodies from each, were tested. This makes a total of 19 analysed fruitbodies.

In the abnormal fruitbody of type I apparently all 3 nuclei recombined. A thorough inspection of the gills from which the sample of basidiospores was taken and an analysis of a larger number of single spore isolates (72 were tested) indicated that probably a mixed sample

Table 4
Fruiting ability of pairs of dikaryotic mycelia

Type*		Number of fruitbodies in bottles from transfer:			Total number of	
		first	second	third	bottles	fruitbodies
I	a	7	2	3	3	12
	b	4	7	4	3	15
II	a	1	6	3	3	10
	b	2	5	1	3	8
III	a	5	4	3	3	12
	b	1	2	2	3	5
Total		20	26	16	18	62

* Cf table 1

of gills originating from 2 different fruitbodies was taken. In this way the total of analysed fruitbodies increased to 20 (table 5).

Table 5
Analysis of fruitbodies

Type*	Nuclei taking part in the formation of the fruitbody						Total number of fruit-bodies
	two nuclei originating from one component dikaryon with requirement of:			two nuclei originating each from a different dikaryon with requirement of:			
	adenine	methionine	choline	adenine	methionine	choline	
I		8	×	×	×	1**	9
II	1	×		×	2	×	3
III	×	8***			×	×	8
Total	1	16			2	1	20

* Cf. table 1.

** Fruitbody from the mixed sample of gills.

*** All isolates from one of the fruitbodies required both adenine and methionine for growth.

In the abnormal fruitbody of type III over 90% of spores germinated, but out of 64 basidiospore isolates tested all required both adenine and methionine for growth. Moreover some of them required choline. The mating type was determined in 8 isolates. The alleles: $A_2 A_5 B_1 B_6$ were found in the isolates, the allele A_5 appearing only once. The requirement of choline was linked with B_6 . It follows from these data that in 7 out of 8 examples tested, strains requiring adenine contained the A_2 allele, although — basing on the characteristic of the original dikaryons — it was expected that A_5 will be closely linked with adenine requirement.

In the remaining 7 fruitbodies from combinations of type III the abnormal reaction did not appear.

Summarised results of the analysis of fruitbodies are presented in table 5. From these results the following conclusions may be drawn:

1. Fruitbodies are most easily formed by dikaryotic mycelia requiring for growth methionine.
2. In mixed cultures fruitbodies may be obtained from dikaryotic mycelia requiring adenine for growth.
3. Fruitbodies may be formed from recombinant dikaryotic mycelia. Such fruitbodies were obtained from recombinant mycelia requiring methionine (2 cases) and requiring choline (1 case).

FORMATION OF RECOMBINANT DIKARYONS ON SUPPLEMENTED MEDIA

Blocks of ca 3 mm. side were taken from dense areas of plates from transfer 1 and transfer 3, grown on complete medium, and inoculated to supplemented media. Media were selected in such a way, that only recombinant dikaryotic mycelia would be able to grow on them singly. The plates were incubated for 6 days and then blocks of ca 1 mm side were taken from the marginal parts of the colonies to check their growth requirements. If the testing demonstrated, that no or very few recombinant dikaryons were produced, new plates were inoculated and grown for 6 days (transfer 2). In the same way transfer 3 was made, if necessary. The results are presented in table 6. Recombinant dikaryons requiring adenine for growth were formed very quickly and already from transfer 1 only recombinant mycelia were obtained. Recombinant dikaryons requiring methionine were obtained in 7 out of 12 examples in transfer 1 and in all but one example in transfer 2. No choline requiring recombinants were obtained in 3 successive transfers.

Two recombinant dikaryons requiring methionine and two requiring adenine were taken for a more detailed analysis. Each was grown on a separate plate supplemented with methionine and adenine respectively and shown to be able to cover the plate with a more or less normal mycelium (Plate I, fig. 5). They were also inoculated to dung bottles. The methionine requiring recombinants fruited and spore analysis demonstrated the expected recombinations of growth requirements and mating type loci. The adenine requiring recombinants were unable to fruit. They were therefore crossed with two wild type strains compatible respectively with one and the other of the expected component nuclei of the recombinant dikaryons. Fruitbodies were obtained from these crosses and spore analysis demonstrated in each case the expected recombinations of growth requirements and mating type loci.

Table 6
Growth requirements of pairs of dikaryotic mycelia grown on supplemented media

Type*	Source transfer	Medium supplemented with	Transfer 1			Transfer 2			Transfer 3		
			number of isolates with requirement of								
			a	m	c	+	+	+	a	m	c
I	a	c	3	—	—	—	—	1	2	—	—
	3	c	3	—	—	—	—	3	2	—	—
	1	c	3	—	—	—	—	1	3	—	—
	3	c	3	—	—	—	—	—	3	—	—
II	1	m	—	—	—	3	—	—	1	—	—
	3	m	—	2	—	1	—	—	—	—	—
	1	m	—	3	—	—	—	—	—	—	—
	3	m	—	2	—	1	—	—	—	—	—
III	1	a	3	—	—	—	—	×	×	×	×
	3	a	3	—	—	—	—	×	×	×	×
	1	a	3	—	—	—	—	×	×	×	×
	3	a	3	—	—	—	—	×	×	×	×

* Cf. table 1.
 ** Able to grow on minimal medium.
 × the transfer was not made.

GROWTH OF PAIRS OF DIKARYOTIC MYCELIA ON MINIMAL MEDIUM

Paralell to inoculations to complete medium, described previously inoculations were made in the same way to plates of minimal medium. As before three successive transfers were made, each growing for 6 days. The growth of mycelium was usually irregular, but quite considerable. Examples of such mycelia, after 6 days of growth on minimal medium are presented in plate I, fig. 6—9.

Table 7

Growth requirements of fainter growing mycelium

Type*		Number of isolates requiring			
		a	m	c	+**
I	a	9	—	—	—
	b	8	—	—	1
II	a	9	—	—	—
	b	5	1	—	3
III	a	4	1	1	3
	b	2	—	—	7
Total		37	2	1	14
among them transfer:	first	13	1	1	3
	second	13	—	—	5
	third	11	1	—	6

* Cf. = table 1.

** Able to grow on minimal medium.

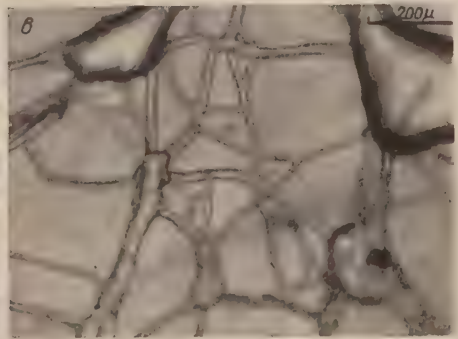
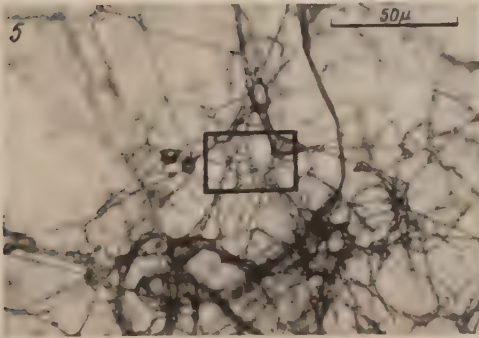
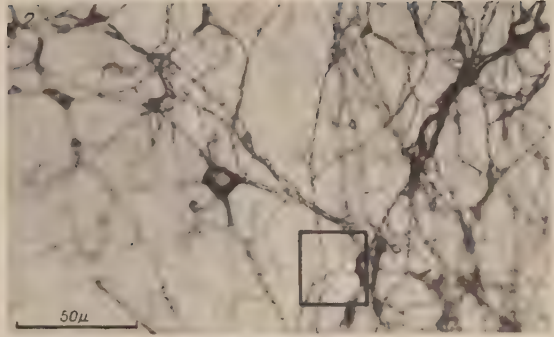
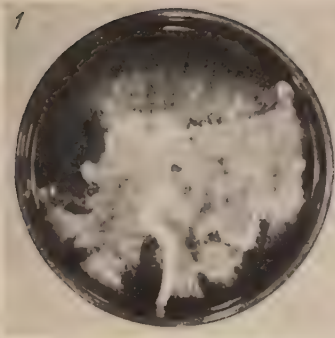
Inocula taken from dense parts of mycelium, used for making successive transfers, always produced growth on minimal medium. In most colonies areas with fainter growth of mycelium could also be detected. The results of the checking growth requirements of the fainter mycelium are presented in table 7. Isolates requiring adenine largely predominate, also in combinations of type III, where such mycelia are expected to contain recombinant dikaryons. Occasionally mycelia with other requirements are present, in one case also a mycelium with choline requirement.

Minimal plates from transfer 3 were kept in the incubator for a prolonged period of time. In one of the plates a rudimentary fruitbody developed (Plate II, fig. 1) indicating that a joint growth of dikaryotic auxotrophic mycelia is able to produce conditions necessary for fruiting, even on minimal medium.

Plate II

- Fig. 1. — A rudimentary fruitbody developed on minimal medium in a plate inoculated with a pair of complementary auxotrophic dikaryotic mycelia
- Fig. 2. — Network of aerial hyphae, a few millimeters beyond the margin of a 6 days old mixed dikaryotic colony. Many pairs and groups of hyphae sticking together visible. The area enclosed by the rectangle magnified on fig. 3
- Fig. 3. — Two hyphae sticking together in aerial dikaryotic mycelium. Morphological changes and the line along which the hyphae appear to fuse is clearly visible. Arrows indicate places where clamps are present
- Fig. 4. — Network of hyphae from the same mycelium as Fig. 2. Places with many hyphae sticking together indicated by arrows
- Fig. 5. — Network of hyphae from the same mycelium as fig. 2. Morphology of the thickened part of mycelium visible. The area enclosed by the rectangle magnified on fig. 6
- Fig. 6. — Network of hyphae, shown on fig. 5, magnified. Several pairs of hyphae sticking together visible
- All pictures taken from living mycelium, growing in Petri dishes.
Picture reproduced on fig. 1 made by S. Kowalski
Pictures reproduced on fig. 2—6 made by Z. Klawender

Plate II



SINGLE HYPHA ANALYSIS OF MYCELIA GROWING ON MINIMAL MEDIUM

From each of the 3 transfers growing on minimal medium, after 6 days of growth, 18 samples containing a few marginal hyphae were isolated from each plate. They were taken from areas with most vigorous, either submerged or surface growth. The samples were transferred to minimal medium. After 24 hours new growth started from the inocula from which single hyphae could be isolated, sometimes branched and up to 2 mm. long. After the single hyphae were isolated, the inocula were allowed to grow for 4 days, and the number of colonies able to develop was scored. The results are presented in table 8. It appears that in all combinations of pairs of mycelia isolates containing only a few hyphae may grow sometimes on minimal medium.

Table 8

Ability to grow on minimal medium of samples containing a few hyphae

Type*		Number of samples	
		isolated	growing on minimal medium
I	a	54	1
	b	54	2
II	a	54	3
	b	54	18
III	a	54	5
	b	54	6
Total		324	35
Among them transfer:	first	108	7
	second	108	17
	third	108	11

* Cf. table I.

The single hyphal isolates were transferred to minimal plates and incubated for 4—6 days. Altogether 108 single hyphal tips were isolated, 41 originating from dikaryon combinations of type I, 36 from type II and 31 from type III. None of them developed into colonies able to grow on minimal medium.

Another 54 hyphal tips were isolated from samples of dense mycelia from colonies grown on complete medium. None grew on minimal medium.

The single hyphal isolates did not show normal growth, but they developed a faint mycelium, typical for auxotrophic mutant strains. A sample of such colonies was taken to check the growth requirement and the presence of clamps. The results are presented in table 9. All the isolates required either adenine or adenine and choline. In all colonies

requiring only adenine clamps were present. All colonies requiring both adenine and choline had no clamps.

The mating types of the colonies requiring both adenine and choline were determined. All colonies originating from combinations of type I had the mating type $A_5 B_5$, all colonies from type II had the mating type

Table 9
Growth requirements of single hyphal isolates

Type*		Number of isolates requiring:		
		a	ac	total
I	a	10	1	11
	b	14	1	15
II	a	11	1	12
	b	8	—	8
III	a	7	2	9
	b	6	5	11
Total		56	10	66

* Cf. Table 1.

$A_5 B_6$ and all colonies from type III had the mating type $A_6 B_5$. This result was expected on the assumption that these colonies are monokaryotic and the nuclei present in them are unchanged component nuclei of one of the original dikaryons (cf. table 1).

MICROSCOPIC OBSERVATIONS ON JOINTLY GROWING DIKARYOTIC MYCELIA

To get some idea, how cytoplasmic connections between dikaryotic mycelia might be established, pairs of such mycelia, growing on plates with complete medium, were observed microscopically. On the agar surface in the periphery of the colonies only single hyphae could be seen, but in aerial mycelium, reaching frequently the colony margin, pairs or groups of hyphae sticking together could be seen (Plate II fig. 2—6). Morphological changes in hyphae sticking together and — partial at least — disappearance of the walls separating them indicates that longitudinal hyphae fusions take place. This picture was observed in monokaryotic and in dikaryotic mycelia, singly or in combinations. Sometimes such aerial hyphae, fused or not, grow beyond the hyphae developing on agar surface, and falling down to the agar, start a marginal surface growth.

DISCUSSION

Three types of combinations of dikaryons were analysed (table 1). In two of them, in which recombinant dikaryotic mycelia required methionine or adenine, the recombinants were obtained several times (tables 5

and 6). Recombinant dikaryotic mycelia with adenine requirement were formed very easily without any selective pressure applied or even against it (tables: 6, 7 and 9). There are indications that recombinant dikaryons may be formed also from choline requiring nuclei (table 5). They were not obtained frequently, because such dikaryotic mycelia are apparently eliminated by mycelia requiring adenine (table 6). It appears from these results that recombinant dikaryons are formed without difficulty. The frequency of their formation largely depends on the genetical constitution of the strains. The cytoplasm did not influence their formation to any large extent in the analysed material.

Two simple ways of forming recombinant dikaryons may be suggested:

1. Fusion of hyphae containing different dikaryons and rearrangement of nuclei within the cell. A network of hyphae was observed in dikaryotic mycelia (Plate II, fig. 2—6) in which many hyphal fusions must have taken place and there is no reason to expect that such fusions would be less frequent between hyphae originating from different mycelia. Polykaryotic hyphae are apparently not stable as in over 160 isolated hyphal tips none appeared to contain 3 kinds of nuclei. It must be expected therefore, that if such fused hyphae produce new growth, the number of nuclei per cell must be reduced to one pair, and this pair may be recombinant.

2. Dikaryotisation of monokaryotic (dedikaryotised) hyphae. It has been shown that single hyphae taken either from the original dikaryotic mycelia (table 2) or from their joint growth (table 9) may contain nuclei of one type only. If such monokaryotic cells exist in the colony and if they are dikaryotised by a nucleus of a foreign dikaryon (Buller phenomenon), a recombinant dikaryon will be formed.

The results obtained do not allow the rejection of either of the alternatives, nevertheless the first appears to be the more likely one. Hyphal fusions are formed in large numbers in aerial mycelium and this mycelium frequently reaches the periphery of the colony, making the marginal growth of recombinants possible. On the other hand monokaryotic hyphal tips appear to be formed most frequently (or exclusively?) under the surface of the agar. Hyphal fusions appear to be less likely there, even for mechanical reasons. Moreover monokaryotic hyphal tips appear to have lost the ability to accept compatible nuclei found in the same hypha (Świeżyński 1961). It may be expected that the acceptance of nuclei from foreign hyphae may also be difficult.

Published data concerning *Coprinus* and other *Basidiomycetes* indicate that both mechanisms may operate in bringing about exchange of nuclei between dikaryons. Hyphae fuse in fungi very frequently (Buller 1933) and Hein (1930) observed in *Psalliota campestris* also longitudinal

hyphal fusions. Studies of Nelson (1956) and Nelson et al. (1954, 1955) indicate that in rusts a polykaryotic mycelium is an intermediate stage in formation of recombinant dikaryons. On the other hand the splitting of dikaryons bringing about the formation of monokaryotic mycelia has also been observed in many fungi. It was found in connection with periodic changes of mycelium, formation of oidia, differentiation of hyphae in fruitbodies and germination of basidiospores of "homothallic" species (Papazian 1950, 1958, Ashan 1952, Terra 1955, older literature in reviews of Buller 1941 and Raper 1953).

There are several indications that in compatible and incompatible di — mon matings exchange of genetic material between nuclei may occur (Crowe 1960, Kimura 1953, review of older data, Papazian 1958). If recombination of this kind had occurred in the material analysed, it would be expected to bring about two kinds of changes:

1. The formation of dikaryotic mycelia able to grow on minimal medium. For instance in the first dikaryon of type I (Table 1) a recombinant nucleus of the constitution $A_2 B_6 m$ might be formed. Such a nucleus would be compatible with the nucleus $A_5 B_5 ac$ of the other dikaryon and in this way a recombinant dikaryon might be formed, able to grow singly on minimal medium. Hyphal tip isolates with such dikaryons would also be able to grow on minimal medium. No such dikaryons were ever observed, although the cultures have been kept for three successive transfers on minimal and complete media and then some were kept for prolonged periods on supplemented media.

2. Formation of recombinant abnormal nuclei. In the analysed material only one case of abnormal reaction was observed i.e. the appearance of additional requirement of adenine. This was found in some hyphal tip isolates originating from one of the dikaryotic mycelia (table 2) and in the progeny of one fruitbody formed by a mixed growth of mycelia, the same dikaryotic mycelium being one of the components (Table 5). This phenomenon does not appear to be due to exchange of genetic material between nuclei. However no satisfactory explanation of this result could be found and a separate analysis of the case is intended.

These results suggest that in dikaryons of *C. lagopus* singly or in joint growth the tendency to somatic exchange of genetic material between nuclei does not exist or is very weak. This may be an indication, that such somatic recombinations are linked with migration of nuclei, which took place in all described cases of somatic recombination in *Holobasidiomycetes*. In our experiments, performed only with dikaryotic mycelia, there was no opportunity for nuclear migration.

In the course of experiments two interesting phenomena appeared:

1. Crossfeeding between dikaryotic mycelia. Single, auxotrophic

ones were unable to grow on minimal medium, but pairs of them with different growth requirements were able to form large colonies. The crossfeeding was also apparent in fruitbody formation. Single auxotrophic dikaryotic mycelia were unable to fruit or fruited poorly (Table 2). On the contrary pairs of such mycelia with complementary growth requirements fruited profusely (Table 4). In single cultures dikaryotic mycelia requiring adenine for growth were never observed to fruit, but such a fruitbody was obtained in joint growth of mycelia (Table 5). The cross-feeding may also be important in fruiting of wild type mycelia. Difficulties in obtaining fruitbodies from single dikaryotic mycelia (Terra 1953) may be due to lack of such crossfeeding.

2. Combinations of nuclei advantageous for dikaryotic growth are not necessarily the same as those most favorable for fruitbody formation. Throughout the whole course of these experiments it was found, that dikaryons composed of nuclei with adenine requirement are most stable (irrespective of nutrient medium used). On the contrary the best fruiting was observed regularly in dikaryons auxotrophic for methionine.

It appears likely that the exchange of nuclei has an evolutionary significance, making possible the formation, multiplication and fruiting of the best adapted dikaryotic mycelia. It may be of special importance in parasites in which monokaryotic and dikaryotic mycelia develop on different hosts. In such parasites it is additionally significant that basidiospores formed on recombinants, and able to produce best combinations, are liberated jointly and have more chance to reach jointly the, frequently distant, alternative hosts.

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SUMMARY

1. From two jointly growing dikaryotic mycelia with mutually compatible nuclei a new dikaryotic mycelium may arise. The component nuclei of the new dikaryon are each derived from a different dikaryon. The frequency of such recombination largely depends on the genetical composition of the nuclei. Its mechanism and possible evolutionary significance are discussed.

2. Jointly growing auxotrophic dikaryons are able to crossfeed. By this means fruitbodies may be obtained from mycelia which are unable to fruit alone.

3. In jointly growing dikaryotic mycelia recombinant nuclei due to internuclear exchange of genetical material were not observed, although such mycelia were grown for prolonged periods of time.

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Biologiczna metoda ilościowego oznaczania kwasu giberelinowego testem pierwszego liścia owsa

A biological assay for gibberellic acid with the first oat leaf

M. MICHNIEWICZ

WSTĘP

Stwierdzenie u roślin wyższych występowania substancji giberelino- podobnych (Radley 1956, 1958, Murakami 1957, Ritzel 1957, Corcoran i Phinney 1958, MacMillan i Suter 1958, Nickell 1958, Phinney i Neely 1958, West i Murashige 1958, Simpson 1958, Kawarada i Sumiki 1960) wywołało konieczność wynalezienia odpowiednich metod ich ilościowego oznaczania.

Metody chemiczne zarówno kolorymetryczne, jak i fluorescencyjne nie są dostatecznie czułe dla oznaczania tak znikomych ilości tych substancji, jakie występują w roślinach wyższych (Overbeek i współautorzy 1957, Kavanagh i Kuzel 1958, Simpson 1958, Henderson i Graham 1960). Ponadto substancje giberelinopodobne, wyekstrahowane z materiału roślinnego, nie zawsze są identyczne z preparatami, jakimi dysponujemy w chwili obecnej w stanie krystalicznym (Henderson i Graham 1960). Dotychczas wyodrębniono bowiem z roślin wyższych substancję identyczną tylko z gibereliną A₁ (Phinney i Neely 1958, MacMillan i Suter 1958, West i Murashige 1958, Kawarada i Sumiki 1960).

Znacznie czulsze są natomiast metody biologiczne, oparte na stymulującym wpływie gibereliny na wzrost pędów. Pozwalają one ponadto na stwierdzenie obecności każdej substancji o własnościach gibereliny.

Niewątpliwie najbardziej niezawodnymi z tych metod są te, w których jako testu używa się całych roślin. Tak np. test karłowatych mutantów kukurydzy reaguje na kwas giberelinowy już w ilości 0,0001 µg na roślinę, a w granicach od 0,001 do 10 µg umożliwia ilościowe oznaczanie tego związku (Neely i Phinney 1957). Podobnie reagują siewki karłowatego grochu (MacComb i Carr 1957).

Możliwości stosowania tych metod są jednak ograniczone konieczno-

ścią posiadania szklarni, a poza tym wyniki uzyskujemy tu po stosunkowo długim okresie czasu.

Znaczne uproszczenie stanowi niewątpliwie metoda Aytouna i współautorów (1959), w której test stanowią siewki ryżu. Rośliny te zanurza się korzeniami w badanych roztworach i po pewnym czasie mierzy się przyrost łodygi. Test ten jest jednak mniej czuły, reaguje bowiem dopiero na giberelinę w stężeniu $0,1 \mu\text{g/ml}$ i wymaga stosunkowo dużej ilości badanego roztworu (8 ml).

Bardzo wyraźnie reagują na giberelinę nie tylko całe rośliny, ale także i niektóre organy: liścienie ogórka (Kribben 1957), liście, np. *Raphanus* (Kuraishi i Hashimoto 1957), a nawet ich fragmenty, np. krążki wycięte z etiolowanych liści fasoli (Scott i Liverman 1957), dalej odcinki etiolowanych pędów grochu (Purves i Hillman 1959), czy wreszcie odcinki wąsów ogórka (Galun 1959).

Jako testy na giberelinę najbardziej przydatne, okazały się jednak przede wszystkim odcinki pierwszego liścia traw — jęczmienia (Hayashi i Murakami 1954), owsa (Hayashi i Murakami 1954, Harada i Nitsch 1959) i pszenicy (Radley 1958). Metody, które tu stosuje się, przypominają w zasadzie test cylindryczny Bonnera (Audus 1959) z tą różnicą, że uwzględnia się przyrost odcinka pierwszego liścia, a nie koleoptyla, jak to ma miejsce przy oznaczaniu substancji wzrostowych typu auksyny.

Testy te nie są jednak wystarczająco czułe. W doświadczeniach Radley (1958) na przykład liście pszenicy reagowały na stężenie kwasu giberelinowego o stężeniu $0,01 \mu\text{g/ml}$, a różnice w intensywności reakcji wzrostowych dla poszczególnych stężeń, różniących się pomiędzy sobą dziesięciokrotnie, okazały się nieistotne. Test ten, a także testy odcinków pierwszego liścia jęczmienia i owsa opisane przez Hayashi i Murakami (1954) nie są przy tym specyficzne tylko dla gibereliny, lecz reagują również na auksynę.

Podobne wady ma także metoda oparta na pomiarach przyrostu odcinków wąsów ogórka (Galun 1959), a poza tym kłopotliwa wydaje się także uprawa tych roślin.

Doświadczenia Radley (1958), Purvesa i Hillmana (1958) wykazały, że badane fragmenty liści, czy też łodygi reagowały tym silniej na giberelinę, im bliższe było ich położenie w stosunku do merystemu szczytowego. Również Vlitos i Meudt (1957) oraz Brian i Hemming (1958) stwierdzili, że fragmenty pędów, w których zachowano przy cięciu tkankę merystematyczną, są bardziej wrażliwe na działanie gibereliny aniżeli odcinki nie zawierające merystemów. Podobną zależność obserwowali Bojarkin i Dmitriewa (1959), także odnośnie reakcji liści na ten preparat.

Spostrzeżenia te dały podstawę Bojarkinowi i Dimitriewie (1959) do opracowania nowego zmodyfikowanego testu liściowego na giberelinę, w którym roślinę testową stanowią młode siewki kukurydzy o przebitych koleoptylach. Siewki takie ucinają się powyżej i poniżej węzła tak, że uzyskane w ten sposób odcinki mają zarówno część koleoptyla z liściem, jak i górną część pierwszego międzywęzła. Odcinki te umieszcza się międzywęzłem do naczynek z badanym roztworem, a następnie mierzy się przyrost pierwszego liścia.

Metoda ta jest bardzo prosta, szybka i wymaga małej ilości roztworu tak, że mogłaby znaleźć zastosowanie w badaniach chromatograficznych i elektroforetycznych materiału roślinnego. Ważny przy tym jest fakt, że test ten reaguje na auksynę tylko w małym stopniu i to hamowaniem wzrostu. Czułość tej metody nie jest jednak duża, umożliwia bowiem ilościowe oznaczanie gibereliny w granicach stężeń od 0,2 do 1,0 $\mu\text{g/ml}$.

W badaniach chromatograficznych i elektroforetycznych materiału roślinnego chodzi jednak zwykle nie tylko o oznaczenie związków giberelinopodobnych, ale także o rozdział poszczególnych regulatorów wzrostu, zwłaszcza związków indolowych, które określa się w tym przypadku głównie przy pomocy testu owsianego. Wydaje się więc bardziej celowe stosowanie testu owsianego także przy oznaczaniu gibereliny.

Konieczne jest również, z uwagi na znikome ilości związków giberelinopodobnych, jakie uzyskuje się przy rozdziale chromatograficznym i elektroforetycznym substancji aktywnych zawartych w materiale roślinnym, podjęcie prób mających na celu zwiększenie czułości tego prostego testu biologicznego.

Dla osiągnięcia tego uzasadnione wydaje się zbadanie wpływu czynników środowiska zewnętrznego warunkujących reakcję rośliny na giberelinę oraz odpowiednie przygotowanie materiału testowego pozwalające na maksymalne zwiększenie jego czułości.

Przebadanie więc przydatności owsianego testu liściowego do ilościowego oznaczania gibereliny oraz ustalenie najbardziej optymalnych warunków wzrostu owsa, pozwalających na uzyskanie najwyższego stopnia czułości testu, było celem niniejszej pracy.

OPIS METODY OZNACZANIA GIBERELINY PRZY POMOCY TESTU PIERWSZEGO LIŚCIA OWSA

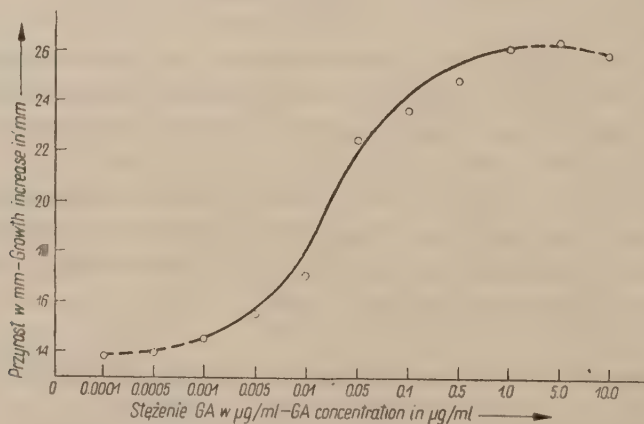
Test owsiany okazał się bardzo przydatny do ilościowego oznaczania gibereliny, a wyniki szeregu doświadczeń opisanych w dalszej części niniejszej pracy pozwoliły na osiągnięcie znacznej czułości rośliny na tę substancję wzrostową.

Szczegóły metody przedstawiają się następująco.

Materiał testowy uzyskuje się tu tak samo jak przy oznaczaniu auk-syny. Nasiona owsa odmiany „Zwycięzca“ (Svalöf), wyselekcjonowane uprzednio pod względem wielkości i moczone przez dwie godziny na świetle, wysiewa się do wilgotnych trocin i kiełkuje w ciemności w temperaturze około 25°C , w atmosferze nasyconej parą wodną do około 95%. Po czterech dniach kiełkowania, gdy tylko koleoptyle zostaną przebite, rośliny pozostawia się przez 6 godzin w świetle dziennym, a następnie w tych samych warunkach świetlnych tnije się je na odcinki obejmujące fragment tkanki 20 mm nad i 5 mm pod węzłem. Istotne jest, aby wszystkie rośliny testowe użyte w doświadczeniu były dobrze wyrosnięte, zwłaszcza zaś, aby były w tej samej fazie wzrostu.

Odcinki zawierające dolną część koleoptyla z pierwszym liściem oraz górną część pierwszego międzywęźla umieszcza się międzywęźlem w ma-lutkich probówkach z $1/2$ —1 ml badanego roztworu. Do każdej próbówki wkłada się po 12 takich odcinków. Roztwory przygotowuje się na 2% glukozie.

Inkubację prowadzi się w ciemności, w temperaturze 30°C przez okres doby, a następnie mierzy się przyrost pierwszego liścia. Przyrost określa się na podstawie pomiaru 10 odcinków z każdej próbówki odrzucając po 2 odcinki skrajnej wielkości najbardziej odbiegające od średniej. Celem uzyskania bardziej miarodajnych danych, doświadczenie wykonuje się w dwu powtórzeniach, uzyskując ostateczny wynik na podstawie 20 pomiarów.



Ryc. 1. Wzrost pierwszego liścia owsa w zależności od stężenia kwasu giberelinowego; linia ciągła — różnice istotne; linia przerywana — różnice nieistotne

The growth of the first oat leaf in relation to the concentration of gibberellic acid; the continuous line — significant differences; the broken line — lack of significant differences

Tabela 1 — Table 1

Wzrost pierwszego liścia owsa w zależności od stężenia kwasu giberelinowego

The growth of the first oat leaf in relation to the concentration of gibberellic acid

Przyrost liścia w:		Stężenie GA w $\mu\text{g/ml}$ — GA concentration in $\mu\text{g/ml}$									
Growth in-	0	0,0001	0,0005	0,001	0,005	0,01	0,05	0,1	0,5	1,0	5,0
crease of leaf in:											
mm	13,07	13,86	13,98	14,52	15,47	17,05	22,42	23,62	24,87	26,07	26,25
‰	100	106,04	106,96	111,09	118,36	130,45	171,53	180,71	190,28	199,46	200,84
											197,39

Najmniejsza różnica udowodniona — The least signif. differ.:

 $P_{0,1} = 0,783$ $P_{0,05} = 0,933$ $P_{0,01} = 1,127$

Przykładowe wyniki z doświadczeń nad zależnością wzrostu pierwszego liścia owsa od stężenia kwasu giberelinowego (Kyowa Fermentationindustry Co., LTD. Tokio) uzyskane przy pomocy wyżej opisanej metody zebrane są w tabeli 1 i przedstawione graficznie na wykresie ryc. 1.

Dane te wskazują, że test reaguje już na kwas giberelinowy w stężeniu $0,0001 \mu\text{g/ml}$, jednak różnica w stosunku do kontroli istotna jest w tym przypadku dopiero przy $P = 0,1$, tj. przy dopuszczalnym błędzie $= 10\%$.

Najniższe stężenie gibereliny, przy którym ujawnia się różnica istotna przy $P = 0,05$ wynosi tu $0,001 \mu\text{g/ml}$.

Istotne różnice przy $P = 0,05$ występują także pomiędzy wariantami różniącymi się pięciokrotnym stężeniem gibereliny. Tylko w przedziałach stężeń od $0,01$ do $0,05 \mu\text{g/ml}$ dadzą się ujawnić różnice mniejsze.

Widzimy także, że w miarę wzrostu stężenia kwasu giberelinowego przyrost liści zwiększa się, osiągając maksymalną wartość w roztworze o stężeniu $1,0\text{--}5,0 \mu\text{g/ml}$; w wyższych stężeniach przyrost ten maleje.

Wyżej opisana metoda pozwala zatem na ilościowe oznaczanie gibereliny w granicach stężeń od $0,001$ do $1 \mu\text{g/ml}$, co stawia ją w rzędzie najbardziej czułych metod określania tej substancji wzrostowej.

WPLYW AUKSYNY NA WZROST PIERWSZEGO LIŚCIA OWSA

Wśród naturalnych regulatorów wzrostu roślin, obok związków gibberlinopodobnych, występuje przede wszystkim auksyna — kwas indoliloctowy i jego pochodne. Istotne jest więc stwierdzenie, czy i w jakim stopniu test na giberelinę reaguje na auksynę.

Tabela 2 — Table 2

Wpływ auksyn na wzrost pierwszego liścia owsa
The effect of auxin on the growth of the first oat leaf

Stężenie IAA w $\mu\text{g/ml}$ — IAA concentration in $\mu\text{g/ml}$						
	0	0,001	0,01	0,1	1,0	10,0
Przyrost liścia w mm	12,45	13,10	12,30	11,80	11,05	11,00
Growth increase of leaf in mm						

Najmniejsza różnica udowodniona. — The least signif. differ.: $P_{0,1} = 0,914$

$P_{0,05} = 1,095$

$P_{0,01} = 1,460$

Wyniki specjalnego doświadczenia z kwasem indoliloctowym, wykonanego ściśle według metody opisanej w poprzednim rozdziale, zebrane są w tabeli 2. Wskazują one, że substancja ta wywiera tylko bardzo nieznaczny wpływ hamujący na test liściowy, obniżając w sposób istotny przyrost pierwszego liścia dopiero w stężeniu $1,0 \mu\text{g/ml}$.

CZYNNIKI WPLYWAJĄCE NA EFEKTYWNOŚĆ TESTU PIERWSZEGO LIŚCIA OWSA

1. Wielkość odcinków i ilość roztworu

Jak wynika z literatury cytowanej we wstępie, odcinki rośliny tym silniej reagują na giberelinę, im bliższe jest ich położenie w stosunku do merystemu. Zależność tę badano również w niniejszej pracy.

Stwierdzono, że wielkość pierwszego międzywęźla nie ma istotnego wpływu na reakcję rośliny. Istotny wpływ ma natomiast początkowa długość pierwszego liścia (tab. 3). Im odcinki te są krótsze, tym efekt działania gibereliny jest wyższy. Przy bardzo krótkich odcinkach obserwuje się jednak zbyt dużą zmienność w przyroście poszczególnych odcinków w obrębie wariantów. Wielkość odcinków pierwszego liścia nie ma jednak wpływu na granicę czułości testu.

Tabela 3 — Table 3

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od jego długości początkowej

The effect of gibberellic acid on the growth of the first oat leaf in relation to its initial length

Stężenie GA w $\mu\text{g/ml}$ GA Concentration in $\mu\text{g/ml}$	Przyrost liścia w Growth increase of leaf in	Długość początkowa w mm Initial length in mm					
		10		20		30	
		mm	%	mm	%	mm	%
0		8,11	100	13,01	100	17,83	100
0,001		10,16	125,27	14,62	112,37	19,03	106,73
0,01		13,76	169,66	16,94	130,20	21,26	119,23
0,1		16,87	208,01	21,37	164,25	26,34	147,72
Najmn. różn. udowod. The least signif. differ. P = 0,05		2,107	—	1,584	—	1,187	—

Wykazano dalej brak istotnej różnicy w reakcji roślin testowych w zależności od ilości roztworu. Bez znaczenia okazał się również poziom zainurzenia odcinków.

W wyniku tych doświadczeń ustalono, że najbardziej odpowiednie są odcinki uzyskane przez ucięcie kielka 20 mm powyżej i 5 mm poniżej węzła (oddzielającego koleoptyl od pierwszego międzywęźla), a ilość roztworu ustalono na $\frac{1}{2}$ —1 ml.

2. Zawartość cukru w roztworze

Jak wynika z danych z literatury, 2% sacharoza (Nitsch i Nitsch 1956) i 2% glukoza (Wilczyńska 1958) wzmagają czułość testów na auksynę. Stwierdzono także dodatni wpływ sacharozy na efektywność działania gibereliny (Hayashi i Murakami 1957, Purves i Hillman 1959).

Tabela 4 — Table 4

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od zawartości cukru w roztworze

The effect of gibberellic acid on the growth of the first oat leaf in relation to the sugar content in the solution

Stężenie GA w $\mu\text{g/ml}$ GA concentration in $\mu\text{g/ml}$	Przyrost liścia w Growth increase of leaf in		Warianty Variants						Najmn. róż- nica udowod. The least signif. differ. P = 0,05
			bez cukru without sugar		2% glukoza 2% glucose		2% sacharoza 2% saccharose		
	mm	%	mm	%	mm	%	mm	%	
0	13,07	100	13,29	100	13,47	100			1,027
0,001	13,96	106,80	15,38	115,72	14,92	110,76			1,004
0,01	15,92	121,80	17,31	130,24	17,84	132,44			1,285
0,1	20,39	156,00	23,77	178,85	23,60	175,20			1,625

Fakty te znajdują również potwierdzenie w wynikach niniejszej pracy (tab. 4). Wskazują one, że cukier wzmacnia efekt, jaki wywiera giberelina na test pierwszego liścia owsa. Rezultaty, jakie uzyskano przy zastosowaniu 2% glukozy i 2% sacharozy, są zasadniczo podobne.

3. Temperatura

Effekt wzrostowy wywołany działaniem regulatorów wzrostu zależy w znacznej mierze od temperatury (Audus 1959). Czynniki te wpływają również na efektywność działania gibereliny. Wskazują na to Hayashi i Murakami (1957) oraz Neely i Phinney (1957), którzy stwierdzili, że w temperaturze 30°C giberelina stymulowała wzrost w większym stopniu aniżeli w temperaturze pokojowej. Temperatura 30°C okazała się także bardziej odpowiednia dla wywołania silniejszej reakcji wzrostowej odcinków pierwszego liścia owsa przy teście na giberelinę opisanym w niniejszej pracy (tab. 5).

Tabela 5 — Table 5

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od temperatury inkubacji odcinków -

The effect of gibberellic acid on the growth of the first oat leaf in relation to the temperature of incubation of sections

Stężenie GA w $\mu\text{g/ml}$ GA concentration in $\mu\text{g/ml}$	Przyrost liścia w Growth increase of leaf in	Temperatura Temperature			
		20°C		30°C	
		mm	%	mm	%
0		7,16	100	13,35	100
0,001		7,70	107,54	15,40	115,35
0,01		8,79	122,76	16,80	125,84
0,1		9,58	133,79	20,33	152,28

4. Wiek kielków i okres trwania inkubacji odcinków

Reakcja rośliny na regulatory wzrostu zależy niewątpliwie od wieku i fazy rozwoju rośliny (A u d u s 1959).

Tabela 6 — Table 6

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od wieku i fazy wzrostu kielków

The effect of gibberellic acid on the growth of the first oat leaf in relation to the age and the growth phase of shoots

Stężenie GA w $\mu\text{g/ml}$ GA concentration in $\mu\text{g/ml}$	Przyrost liścia w Growth increase of leaf in	Wiek kielków w dniach The age of shoots in days					
		3		4		5	
		Koleoptyl nie przebity non-perforated coleoptile		Koleoptyl ledwie przebity hardly perforated coleoptile		Koleoptyl w dzień po przebicciu coleoptile one day after perforation	
		mm	%	mm	%	mm	%
0		10,70	100	10,65	100	12,55	100
0,001		10,92	102,05	11,89	111,64	13,64	108,68
0,01		12,49	116,72	14,62	137,27	15,85	126,29
0,1		15,00	140,18	20,30	190,61	20,65	164,54
Najm. różnica udowodniona The least signific. differd.	P = 0,1	1,356	—	0,991	—	1,001	—
	P = 0,05	1,622	—	1,185	—	1,198	—

Dane zebrane w tabeli 6 wskazują, że przy oznaczaniu gibereliny testem pierwszego liścia owsa najbardziej przydatne są kielki o zaledwie przebitych koleoptylach. Fazę tę osiągają rośliny po czterech dniach hodowli w temperaturze około 25°C w komorze o 95% wilgotności względnej powietrza. Kielki młodsze i starsze słabiej reagują na tę substancję wzrostową.

W testach biologicznych ważne jest również ustalenie odpowiedniego okresu, po którym należy określać przyrost rośliny po zadziałaniu preparatem, aby uzyskać najbardziej wyraźne wyniki. W zależności od metody wprowadzania substancji i gatunku rośliny okres ten może być bardzo różny.

Tabela 7 — Table 7

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od długości okresu inkubacji odcinków

The effect of gibberellic acid on the growth of the first oat leaf in relation to the length of incubation period of sections

Stężenie GA w $\mu\text{g/ml}$ GA concentration in $\mu\text{g/ml}$	Przyrost liścia w Growth increase of leaf in		Okres inkubacji w godz Incubation period in hrs.					
			24		48		72	
			mm	%	mm	%	mm	%
0			13,35	100	31,00	100	38,05	100
0,001			15,40	115,35	33,27	107,32	40,35	106,04
0,01			16,80	125,84	38,35	123,70	46,20	121,41
0,1			20,33	152,28	43,50	140,32	52,30	137,45

Wyniki specjalnego doświadczenia przedstawione na tabeli 7 wskazują, że w przypadku testu pierwszego liścia owsa, najwyraźniejszy efekt wzrostowy osiąga się po 24 godzinach inkubacji odcinków. Krótszych okresów czasu nie uwzględniano, z uwagi na konieczność objęcia doświadczeniem całego cyklu dobowego rytmu wzrostu (B ü n n i n g 1953).

5. Wiek nasion

Jak wynika z pracy Tyce (1957) pochwki liściowe wyrosłe z nasion starszych są mniej czule na kwas indoliloctowy aniżeli rośliny wyrosłe z nasion pochodzących z wcześniejszego zbioru.

Tabela 8 — Table 8

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od wieku nasion.
(Dośw. wykonane w październiku 1960 r.)

The effect of gibberellic acid on the growth of the first oat leaf in relation to the age of seeds
(Experiment period — October 1960)

Przyrost liścia w Growth increase of leaf in		Rok zbioru nasion The year of harvesting					
		1957		1958		1959	
		mm	%	mm	%	mm	%
Stężenie GA w $\mu\text{g/ml}$ GA concentration in $\mu\text{g/ml}$							
0		14,03	100	14,00	100	11,94	100
0,001		15,10	107,62	15,30	109,23	13,33	111,64
0,01		15,21	108,41	16,26	116,14	15,41	129,06
0,1		23,05	164,29	21,36	152,57	23,44	196,31
1		26,21	186,81	26,63	190,21	27,33	228,89
Najmn. różn. udowod.	P = 0,1	1,103	—	1,206	—	1,003	—
The least significant differ.	P = 0,05	1,319	—	1,442	—	1,199	—

Zależność tę zbadano także w niniejszej pracy. Dane zebrane w tabeli 8 wskazują, że kielki owsa wyrosłe z nasion starszych reagują na giberelinę słabiej.

6. Odczyn roztworu

Pobieranie regulatorów wzrostu przez roślinę uzależnione jest w znacznym stopniu od odczynu środowiska. Hansen i Bucholtz (1952) oraz Hauser (1955) stwierdzili to odnośnie do kwasu 2,4-D, a wyniki nie opublikowanych dotychczas prac, prowadzonych w Zakładzie Fiziologii Roślin U.M.K., wykazały taką samą zależność w przypadku kwasu indoliloctowego.

Fakt różnej reakcji rośliny na substancje wzrostowe w zależności od odczynu środowiska brany jest pod uwagę w testach biologicznych na auksynę. Celem utrzymania najbardziej odpowiedniego pH roztworu stosuje się tu często bufory (Nitsch i Nitsch 1956).

W niniejszej pracy uwzględniono także to zagadnienie, badając wpływ pH roztworu na reakcję pierwszego liścia owsa na giberelinę. Jak widać z danych zebranych w tabeli 9, reakcja ta jest w dużym stopniu nie-

zależna od odczynu roztworu. Tylko w roztworze najbardziej kwaśnym (pH 1,7) i w mniejszym stopniu w roztworze najbardziej zasadowym (pH 11,0) reakcja testu na giberelinę jest wyraźnie słabsza.

Wyniki doświadczenia tego wskazują więc, że stosowanie buforów w teście pierwszego liścia owsa na giberelinę jest niecelowe.

7. Światło

Szybkość wzrostu rośliny jest w znacznym stopniu uzależniona od światła. Są dane, które wskazują, że wpływa ono w znacznym stopniu na poziom i przemieszczanie się kwasu indoliloctowego w roślinie. Wiadomo także, że światło może wywołać fotochemiczną inaktywację auksyny (Strebecko 1956).

Wiele autorów wykazało także współzależność w działaniu światła i gibereliny na wzrost rośliny (Curry i Wassink 1956, Lockhart 1956, 1958a, b). Konieczne wobec tego było przebadanie reakcji wzrostowej pierwszego liścia owsa na giberelinę, w zależności od warunków świetlnych, zarówno w okresie poprzedzającym zadziałanie preparatem, jak i w momencie jego działania.

Doświadczenie mające na celu wyjaśnienie tego zagadnienia prowadzono w luminostatach, na świetle białym zbliżonym składem do naturalnego, o intensywności promieniowania na wysokości roślin około $3500 \mu\text{W}/\text{cm}^2$ (około 6000 lux).

Działanie światłem w okresie inkubacji odcinków nie wpływało w sposób istotny na reakcję testu na giberelinę. Wyraźny efekt wzrostowy zaobserwowano natomiast w przypadku naświetlania roślin, przed cięciem kielków.

Dane obrazujące tę zależność zebrane są w tabeli 10. Wskazują one, że naświetlanie roślin w okresie poprzedzającym cięcie potęguje działanie gibereliny, przy czym najlepszy efekt daje naświetlanie 6-godzinne. W wyniku tych doświadczeń stwierdzono dalej, że wszystkie prace związane z przygotowaniem testu pierwszego liścia mogą być wykonywane na świetle.

WNIOSKI

Opisano metodę ilościowego oznaczania gibereliny przy pomocy testu pierwszego liścia owsa. Wszystkie szczegóły metody opracowano na podstawie wyników specjalnych eksperymentów, w których zbadano wpływ czynników środowiska zewnętrznego warunkujących reakcję rośliny na giberelinę oraz określono sposób przygotowania materiału testowego, pozwalający na zwiększenie jego czułości.

Tabela 9 — Table 9

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od pH roztworu

The effect of gibberellic acid on the growth of the first oat leaf in relation to solution pH

Stężenie GA w $\mu\text{g/ml}$ GA concentration in $\mu\text{g/ml}$	Przyrost liścia w Growth increase of leaf in	pH roztworu pH of solution																			
		1,7		2,5		3,6		3,9		4,4		5,6		6,3		7,7		9,7		11,0	
		mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%
0		13,35	100	12,05	100	11,35	100	12,35	100	11,75	100	12,40	100	13,71	100	12,41	100	13,11	100	12,92	100
0,1		17,10	128,08	21,85	181,32	21,70	191,27	21,91	177,40	21,62	184,00	22,01	177,50	23,28	169,80	22,07	177,84	21,27	162,24	20,31	157,19

pH 1,7 — 4,4 — uzyskano przez dodanie HCl (obtained by adding HCl).

pH 6,3 — 11,0 — uzyskano przez dodanie NaOH (obtained by adding NaOH).

pH roztworu 2% glukozy = 5,6 (pH of 2% glucose = 5,6).

Tabela 10 — Table 10

Wpływ kwasu giberelinowego na wzrost pierwszego liścia owsa w zależności od warunków świetlnych podczas hodowli kielków

The effect of gibberellic acid on the growth of the first oat leaf in relation to the light conditions during the growth of shoots

Przyrost liścia w Growth increase of leaf in Stężenie GA w $\mu\text{g/ml}$ GA concent- ration in $\mu\text{g/ml}$	Czas naświetlania kielków bezpośrednio przed cięciem w godz. The time of lighting of shoots immediately before the cutting, in hrs.													
	0		1		3		6		12		24		48	
	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%	mm	%
0	15,95	100	15,62	100	14,37	100	13,09	100	13,01	100	12,81	100	11,27	100
0,001	16,55	103,76	16,51	105,69	15,55	108,21	14,55	111,09	14,58	112,17	13,48	105,23	11,29	100,17
0,01	19,32	121,12	18,80	120,35	18,17	126,44	17,10	130,45	16,55	127,20	15,95	124,51	14,10	125,11
0,1	24,97	156,55	23,56	150,83	24,11	167,78	23,85	180,71	22,93	176,24	21,16	165,18	18,53	164,24
1,0	27,55	172,72	27,04	173,11	27,28	189,83	25,95	198,24	24,65	189,45	22,83	178,22	19,18	170,18

Przedstawiona w niniejszej pracy metoda pozwala na ilościowe oznaczanie gibereliny w granicach stężeń od 0,001 do 1,0 $\mu\text{g/ml}$, co stawia ją w rzędzie najbardziej czułych metod określania tych substancji wzrostowych. Metoda ta jest przy tym bardzo prosta, szybka i może znaleźć zastosowanie w badaniach chromatograficznych i elektroforetycznych materiału roślinnego. Służyć może także jako bardzo prosty i wygodny test w doświadczeniach fizjologicznych nad współzależnością różnych czynników i gibereliny we wzroście rośliny.

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SUMMARY

A quantitative method of gibberellic acid determination with the first oat leaf is described. All details of the method are based on the results of special experiments. In these experiments the effect of several factors influencing oat leaf sensitivity to gibberellin was investigated namely that of the shoots and seeds age, the sections length, the solutions quantity and its pH, sugar addition, light, the period of incubation of the sections and of temperature conditions.

The details of the method are as follows.

The plant material is obtained in the same way as in the method of auxin determination. The same size oat seeds of Victory variety (Svalöv) soaked in water for 2 hours, in daylight, are sowed in wet sawdust. They are germinated in darkness at about 25°C and at about 95 p.c. air humidity. After 4 days of germination, as soon as the coleoptiles are just pierced, the plants are set for 6 hours in daylight and immediately after they are cut into sections containing parts 20 mm above and 5 mm below the node. All test plants should be in the same growth phase and well developed.

The sections containing the part of coleoptile with the first oat leaf and part of the first internode are put into small test-tubes with $\frac{1}{2}$ —1 ml of determined solutions. The gibberellic solutions are prepared in 2 p.c. glucose. Twelve such sections are put into every test-tube with their basal part down.

The incubation of sections lasts 24 hours at 30°C. After this period the elongation of the first leaf is measured. For the measurement only 10 most typical sections from every test-tube are taken.

In order to make the results more reliable, experiments are repeated twice. In this way definitive results of 20 measurements of sections in every variant are obtained.

The results of gibberellic acid quantity determination obtained by using the method described above are illustrated in the table and in figure 1.

The outcome of the present investigations show a very high sensitivity of first oat leaf test to gibberellin. This test allows to determine gibberellic acid in concentration of 0.001 $\mu\text{g/ml}$ assuming the significant differences at $P = 0.05$ or even in a concentration of 0.0001, at $P = 0.1$. (Details in the table and in figure 1).

The oat leaf test is not sensitive to IAA. The inhibitory effect appears only in high concentration, starting from 1.0 μ g IAA in ml. (Tab. 2).

The biological test for gibberellic acid presented in this paper is very sensitive, simple, quick and can be used in chromatographic and electrophoretic study of plant material. It can be also used as a convenient and simple test in physiological experiments, especially in researches on the interactions of some factors and gibberellin in plant growth.

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Observations on the development of plants IV.

The development of annual hibernating plants

A. LISTOWSKI and I. JACKOWSKA

The influence of exterior conditions (chiefly of light and temperature) on the development of plants appears — with the accumulation of more experimental data — both more complicated and varied than it was so far believed (Bünning 1953, Junges 1958).

Our observations carried on since 1957 on various species of perennial and annual plants, whether hibernating or beginning their vegetation in spring (Listowski and Jeśmianowicz 1960a, 1960b, 1961) also indicate a varying relation between development and exterior conditions as well as different interaction of light and temperature in their inhibiting or stimulating influence on vegetative and generative development. It also results from our observations that the development of many perennial, biannual and hibernating plants is not inhibited but only retarded, if these plants grow continually in elevated temperature conditions, while the influence of daylength extends to all plants, though its intensity may vary.

Further observations are in progress and are being extended to other species both perennial and hibernating.

According to Tymrakiewicz (Weed Atlas, 1959), of the 149 species of our segetal flora 34 are perennials, and above 65 — spring species, germinating, however, mostly in late summer and hibernating in the form of a rosette.

Of this group of hibernating plants the following have been examined (Listowski and Jeśmianowicz 1960a, 1960b): *Centaurea cyanus*, *Agrostemma githago* and *Delphinium consolida*. In all of these a strong inhibiting effect of short day treatment of various intensity was established in dependence on the data of sowing, but practically no stimulating effect of lower temperature was observed.

It seemed of interest to extend observations to further species belonging to this group of plants (Table 1).

Experiments were, also undertaken on three species not included in the above table:

Table 1

Species	Date of sowing	L			S			Differ- ence S — L
		Number of days to:		No. of flower ing plants in %	Number of days to:		No. of flower- ing plants in %	
		begin of flower.	full bloom		begin of flower.	full bloom		
<i>Veronica arvensis</i>	1.VI	42	50	100	44	54	100	4
	6.VII	42	50	100	46	57	100	7
	25.VIII	77	95	100	95	117	100	22
<i>Lithospèrnum arvense</i>	1.VI	—	36	100	—	37	100	1
	1.VII	—	46	100	—	48	100	2
	31.VIII	—	103	100	—	144	100	41
	16.II	42	47	100	63	70	100	23
<i>Viola tricolor</i>	6.VII	—	43	100	—	45	100	2
	7.VIII	168	235	100	240	256	50	21
	31.VIII	158	198	75	198	222	40	24
<i>Arenaria serpyllifolia</i>	1.VI	42	46	100	48	60	100	14
	6.VII	44	56	100	81	100	80	44
	25.VIII	57	77	100	109	136	50	59
<i>Cerastium viscosum</i>	1.VI	56	61	100	79	92	100	31
	6.VII	66	78	100	77	97	80	19
	7.VIII	74	95	100	222	262	100	167
	31.VIII	74	89	100	198	237	80	146
<i>Cerastium vulgatum</i>	31.VIII	80	89	100	203	224	60	135
	16.II	69	74	100	99	—	—	—
<i>Galium aparine</i>	1.VII	59	68	100	—	161	100	23
	25.VII	—	65	100	—	136	100	71
<i>Papaver Rhoeas</i>	23.VII	217	241	100	—	332	100	91
	25.IX	217	227	100	217	—	spor.	—
<i>Capsella bursa pastoris</i>	6.VII	39	50	100	—	78	100	28
	7.VIII	59	76	100	88	111	100	35
	31.VIII	65	72	100	112	125	100	53
<i>Geranium pusillum</i>	9.VII	96	111	100	—	325	100	214

1. *Spergula arvensis* (winter or spring species) exclusively under natural daylight of varying length. Very slow growth in autumn and in the first half of winter. In February extension of shoots. The plants flowered early and died towards the end of April. *Draba verna* (winter species) — complete inhibition of growth under winter and autumn short day of low intensity. From mid January new leaves appear — the plants came into flower early and died after 7 weeks.

2. *Oxalis acetosella*. Sown on August 25. Flowered under continuous daylight after 263 days. Under short day conditions complete inhibition of growth and gradual wilting. Sown on February 16: came into flower under continual daylight conditions after 101 days; under short day conditions but of higher intensity than in autumn flowered after 134 days.

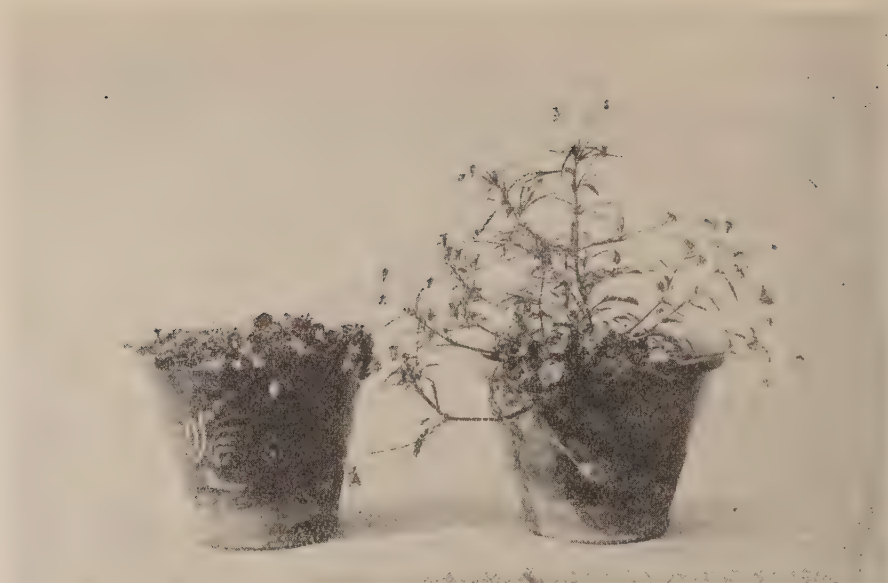
The plants, for which observations are summarized in Table 1, were sown at various dates.

The plants sown in June and July were kept during the whole vegetation period in the vegetation house. Long day (L) means here natural long days gradually becoming shorter. Short day (S) — daylight reduced to ± 8 hours by shading the plants from 3 P.M. The plants sown in the period from August to the first days of September were transferred to the glass-house, placing the combination "L" under continuous illumination and the combination "S" under an 8 hours daylight regime. The intensity of natural light was raised by the use of a fluorescent lamp in combinations "S" and "L" and daylength was prolonged in combination "L".

The minimal temperature in the glasshouse did not descend below 12° and the maximal on sunny spring days reached 30° .

The conditions of development of the plants sown in early summer differed from those in which the autumn-winter series were sown in the hothouse. In spite of the same daylength in the combination "S", the light conditions were different, both as regards intensity and the spectral composition of light. Different were also the temperature amplitudes. This should be kept in mind when comparing the development of plants in series sown at different dates.

Generative development. As may be seen from the data in Table 1. all the plants flowered without vernalization though earlier on a long day regime and at a delayed term under short day. The inhibitive influence of short day on the generative phase — weaker in plants sown in summer — increases greatly, if the plants grow in autumn and winter under less intensive illumination. Under these light conditions the flowering in combination "L" is also delayed. In *Cerastium* and *Arenaria* — in combination "L" — the retardation of development in series sown later



Phot. 1. *Viola arvensis* — sown 6.VII. Left — short day, right — long day, after 100 days of vegetation



Phot. 2. *Capsella bursa pastoris* — sown 6.VII. Left — short day, right — long day, after 100 days of vegetation



Phot. 3. *Cerastium arvense*. From left: short day, sown 7.VIII, long day, sown 7.VIII — after 75 days of vegetation; short day, sown 6.VII, long day, sown 6.VII — after 100 days of vegetation



Phot. 4. *Lithospermum arvense* — sown 31.VII. Left — long day, right — short day, after 190 days of vegetation



Phot. 5. *Lithospermum arvense* — sown 16 II. Left — long day, right — short day. after 53 days of vegetation

is relatively smaller, while in combination "S" the effect is very pronounced.

In *Viola* changes of light conditions cause a marked delay in flowering both in combination "L" and "S", with some of the "S" plants not coming into flower at all (same as *Cerastium* and *Arenaria*).

In plants sown in February the retarding influence of the conditions of combination "S" distinctly decreases (*Lithospermum*, *Cerastium vulgatum*).

In combination "S" the inflorescences are smaller and the number of flowers is reduced; this fall is also observed in combination "L" when sown in autumn. Under continuous illumination both main and lateral shoots come into flower, while in "S" only the main ones do and it is often observed that the flowers develop only successively after the wilting of the preceding ones. In combination "S" the flowering and further development of lateral shoots occurs parallelly, *Lithospermum* only being an exception, as, in combination "S", its lateral shoots develop more intensively and flowering lasts longer and is more intensive.

The differences in coming into flower are illustrated by photographs of some of the plants. Generally speaking, in all species, the strong inhibitive influence of short day conditions in plants sown late is due both to the reduced daylength and to the decreased light intensity. It is prob-

Table 2

Date of measurement		Height of main stem in cm.		No. of leaves on main shoot		No. of lateral shoots		Length of lateral shoots in cm.		Aerial parts of one plant (g)				Root weight of one plant (g)	
Development stage of plants	No. of days from sowing									fresh weight		dry weight		dry weight	
		L	S	L	S	L	S	L	S	L	S	L	S		
Cerastium viscosum — sown 7.VIII															
Flowering of series L	95	30.90	5.1	24	21	7	9	15.1	2.4	2.58	—	0.27	—	—	—
Flowering of series S	270	—	26.3	—	34	—	19	—	21.2	—	15.50	—	2.14	—	—
Cerastium viscosum — sown 31.VIII															
Flowering of series L	90	37.6	4.0	22	17	7	3	—	—	2.48	—	0.39	—	—	—
Flowering of series S	240	—	16.2	—	36	—	16	—	—	—	16.67	—	2.05	—	—
Cerastium vulgatum — sown 31.VIII															
Flowering of series L	90	58.3	8.0	25.0	22	7	4	—	—	7.03	—	0.79	—	—	—
Flowering of series S	230	—	32.4	—	32	—	18	—	—	—	19.30	—	2.46	—	—
Capsella bursa-pastoris — sown 7.VIII															
Flowering of series L	80	30.0	1.4	19	30	—	—	—	—	1.83	—	0.18	—	0.07	—
Flowering of series S	120	—	12.3	—	32	—	—	—	—	—	2.13	—	0.20	—	0.16
Capsella bursa-pastoris — sown 31.VIII															
Flowering of series L	75	191	roz.	13	26	—	—	—	—	2.33	—	0.25	—	0.07	—
Flowering of series S	125	—	6.4	—	29	—	—	—	—	—	2.53	—	0.23	—	0.14
Lithospermum arvense — sown 16.II															
Flowering of series L	50	8.0	1.6	8	6	—	—	—	—	0.45	0.22	0.06	0.02	0.03	0.01
Flowering of series S	70	—	35.6	—	24	—	—	—	—	—	4.62	—	0.54	—	0.06
Veronica arvensis — sown 25.VIII															
Flowering of series L	100	25.6	18.2	20	19	12	10	15.2	9.8	1.71	—	0.40	—	1.18	—
Flowering of series S	120	—	27.0	—	25	—	10	—	14.7	—	0.67	—	0.11	—	0.54

able also that the change in the spectral composition of light plays here a certain role (Wasink and Stolwijk 1956).

Under long day or continuous illumination conditions the individual plants come into flower, end flowering and their seeds ripen almost simultaneously. In combination "S" — on the contrary — the differences in the individual development of the plants are much wider. The life of the entire plant as well as of the particular leaves is significantly prolonged. These differences between "L" and "S" are particularly marked when the plants are sown in autumn. The long day plants sown in July, when transferred to the autumn-winter short day conditions begin to grow and flower again.

Vegetative development. In plants sown earlier (in summer) the differences in vegetative development between series "S" and "L" are relatively small and increase with later dates of sowing.

The reaction in the particular species (with some exceptions) is similar. therefore we have restricted the data given in table 2 to only several species.

The plants of series "S" as compared to "L" exhibit:

1. A reduced rate of growth of the main stem and lateral shoots. In many plants this inhibition is particularly marked in the period from October to January, decreasing later. Growth, particularly in series "S", is visibly accelerated. The stems in this combination are mostly thicker.

2. The rate of formation of lateral shoots varies: it is lower e.g. in *Viola* and *Cerastium* and higher and more intensive in *Lithospermum*.

3. The internodes are distinctly shorter giving the plants, together with the horizontal position of the leaves, a compact habitus, so that the plants often adhere to the ground.

4. The leaves are mostly smaller, rarely of the same size (*Viola*) or larger (*Lithospermum*). Their colour is generally darker, seldom lighter (*Papaver*).

5. The petioles are shorter, the blades smaller (*Veronica*, *Cerastium*, *Capsella*) less frequently larger (*Lithospermum*) or about the same size (*Viola*).

6. The rate of growth of the leaves is slower (e.g. *Cerastium*, *Viola*, *Lithospermum*), the same (*Veronica*, *Arenaria*) or quicker (*Capsella*).

SUMMARY

Observations on some dozen species of monocyclic plants hibernating in our conditions in rosette form, proved a strong dependence of growth and generative development on daylength and light intensity.

Long daylight, notwithstanding the intensity of light, accelerates both vegetative and generative development.

Short daylight retards flowering. Its effect is particularly strong when combined with low light intensity. It results in greatly delayed flowering and its reduced intensity, so that some of the plants do not come into flower at all or develop only single flowers.

Simultaneously a marked retardation of growth processes is observed up to almost complete inhibition of differentiation of leaves but with simultaneous considerable prolongation of the life of the plants as well as of their particular leaves.

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Étude sur la végétation des dunes anciennes en Petite Camargue

*Badania nad roślinnością starych wydm śródlądowych Petite Camargue
w delcie Rodanu*

K. ZARZYCKI

INTRODUCTION

La flore et la végétation de la Grande Camargue sont assez bien connues grâce aux études de Flahault et Combres (1894), Kühnholtz-Lordat (1923) et Tallon (1930—1955); au contraire, la végétation de la Petite Camargue n'a pas encore été étudiée en détail. Quelques données floristiques, d'ailleurs peu nombreuses, se trouvent dans les publications énumérées ci-dessus. Le présent travail, concernant la végétation des dunes anciennes situées à l'Ouest du Petit Rhône, a pour but de combler cette lacune.

Ces dunes, au point de vue de leur âge, forment deux types principaux groupés en deux complexes: les plus anciennes à l'intérieur de la terre ferme — c'est-à-dire le lido Godesque et les dunes plus jeunes de la Petite Camargue. Elles diffèrent également par la flore et la végétation. Leur étude a beaucoup contribué à mieux connaître les stades bien avancés de la succession de la végétation (série de *Ammophilon*) et l'influence du pin parasol (*Pinus pinea*) sur le développement de la végétation et des sols dunales. Dans le but de mieux éclaircir les problèmes de la répartition et de l'immigration de certaines plantes dans les dunes, on a attentivement observé leur distribution non seulement dans les dunes, mais également dans les formations du pliocène, voisinant avec elles et séparées par une bande d'alluvions. L'auteur, au contraire ne s'est pas occupé spécialement de la végétation bien connue du cordon littoral contemporain (Kühnholtz-Lordat 1923, Braun-Blanquet 1952).

Les matériaux pour ce travail ont été recueillis par l'auteur pendant son séjour de trois mois et demi (avril — juillet 1959) à la Station Internationale de Géobotanique Méditerranéenne et Alpine (SIGMA) à Montpellier.

Je remercie cordialement Monsieur le Dr J. Braun-Blanquet, directeur de la Station, de m'avoir indiqué le thème d'étude et pour son aide bienveillante. Je suis également reconnaissant à M. R. Sutter (Montpellier), assistant à SIGMA.

qui a fait avec moi quelques excursions dans le terrain et qui m'a aidé à déterminer les matériaux d'herbier, à M. le Professeur B. Szafran (Cracovie) pour la détermination des échantillons de mousses et à M. J. Nowak (Cracovie) pour celle des lichens.

J'adresse de vifs remerciements à la Deuxième Faculté de l'Académie Polonaise des Sciences pour la bourse qui m'a permis de faire ces études.

REMARQUES MÉTHODIQUES

L'étude de la végétation est basée sur les méthodes phytosociologiques bien connues (Braun-Blanquet 1951).

J'ai observé et décrit les profils des sols dans les groupements étudiés. Des échantillons des sols furent recueillis pour des études laboratoires dont les résultats sont représentés sur le tableau 6.

L'analyse mécanique a été faite d'après la méthode de Cassagrand modifiée par Prószyński. La réaction des sols a été déterminée avec le pH-mètre de production polonaise à l'électrode en verre; la teneur en CaCO_3 en utilisant l'appareil de Passon (en même temps on a vérifié un certain nombre d'échantillons avec l'appareil de Scheibler sans remarquer des différences essentielles). Le contenu total de matière organique a été déterminée par la perte au feu (ca 400°C) et l'humus avec la méthode d'oxydation sulfochromique.

TERRAIN D'ÉTUDES

a. Situation géographique et géologique

Le terrain d'étude comprend les dunes anciennes, d'origine maritime, situées à l'Ouest du Petit Rhône (fig. 1). Ce n'est qu'une petite partie de l'unité géomorphologique représentée par la Camargue. Nous allons la caractériser brièvement en s'appuyant sur les données rassemblées dans l'ouvrage de Galtier (1958—1959, v. aussi Kühnholz-Lordat 1923).

Nous comprendrons sous le nom de Camargue non seulement l'île placée entre les deux bras du Rhône (la Camargue proprement dite), mais aussi la plaine de Saint-Gilles, le Plan du Bourg et la Petite Camargue. La Camargue ainsi conçue doit son existence à l'action combinée de la mer et du fleuve.

Le delta actuel du Rhône a été formé au déclin de la transgression post-würmienne et s'appuie sur un delta pléistocène, qui a eu une plus grande étendue et dont les bords nettement tracés au Nord d'Aiguesmortes sont formés par des cailloutis pliocènes; il descend jusqu'à la profondeur de 200 m au-dessous niveau actuel de la mer.

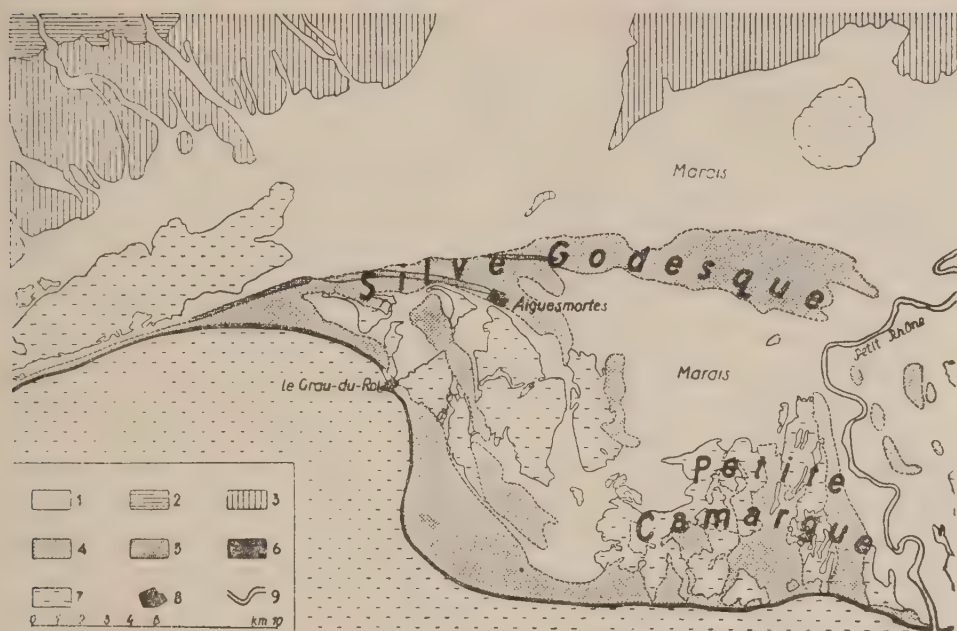


Fig. 1. Carte géologique de la partie occidentale du delta du Rhône (Carte géologique détaillée (de France); feuilles: Montpellier et Arles; un peu schématisé): 1 — alluvions modernes; 2 — éocène supérieur; 3 — pliocène supérieur et moyen; 4 — formations dunaires; 5 — cordons littoraux récents; 6 — cordon littoral moderne; 7 — l'eau (étangs et mer); 8 — villes; 9 — Petit Rhône

Une lente transgression de la mer a commencé au début de la dernière glaciation (würmienne). Environ 5000 ans av. notre ère la mer atteignit la cote -8 mètres. Ensuite elle s'est élevée à la cote -5 mètres et enfin elle stationna à -3 mètres et finit par arriver rapidement à la cote $+1$ mètre. Cela s'est passé environ 3500 ans av. n. e. De cette façon la Basse Camargue limitée par le cordon littoral contemporain est coupée par le reste de l'ancien cordon dunal qui marque la progression de la mer au Nord. Le cordon le plus avancé dans cette direction, qui s'est formé le plus tôt, c'est le lido Godesque. Il apparaît distinctement entre Aiguesmortes et Sylvéréal où il porte le nom de Silve Godesque, qui lui vient de la belle forêt de pins parasols qu'il porta avant d'être défriché. Cette bande de dunes est d'une largeur de 1,5 kilomètre en moyenne et s'étend de l'Ouest vers l'Est à plus de 20 kilomètres. La présence de coquillages marins, identiques à ceux que l'on trouve dans les dunes contemporaines (par exemple *Cardium tuberculatum* et beaucoup d'autres) nombreux dans les dunes Silve Godesque, est une preuve irréfutable de l'origine marine et non fluviale de ces dunes. Celles-ci sont

presque entièrement couvertes de vignobles. Les bois de pin parasol se sont à peine conservés çà et là. Les groupements arbustifs sont mieux développés sur les flancs oriental et occidental (Silve de Montcalm et à l'Ouest du Mas du Garde). Ces dunes atteignent leur hauteur culminante (3—4 mètres) au Nord et au Nord-Ouest d'Aiguesmortes (le vieux cordon littoral, fig. 1). Au fur et à mesure qu'on avance vers l'Est les dunes diminuent de hauteur et finissent par disparaître sous les dépôts alluviaux entre Montcalm et Sylvéréal. Sur la rive gauche du Petit Rhône, près du Château d'Avignon elles réapparaissent à l'état fragmentaire. Au Nord-Ouest du Grau-du-Roi un ancien cordon littoral se lie au cordon contemporain. Il ne prend cependant nulle part contact avec des formations géologiques plus anciennes. Il est séparé d'elles par des dépôts alluviaux couverts d'eau pour la plupart et occupés par des groupements de l'ordre *Phragmitetalia* et *Salicornietalia*. Dans l'endroit où les formations pliocènes s'approchent le plus des anciennes dunes aux environs de Gallician, elles sont toujours séparées au moins par une bande d'alluvions de 4 kilomètres de largeur environ (Marais de la Souteyranne).

Les dunes de la Petite Camargue proprement dite se trouvent au Sud de ce cordon et sont plus jeunes. Elles sont limitées à l'Est par le Petit Rhône et à l'Ouest par le canal de Sylvéréal. Elles ne forment pas un complexe uni sous le rapport morphologique. Elles sont séparées par des étangs plus ou moins grands, salés, de temps en temps visités par des flamants. Les dunes ici sont assez basses en général et quelquefois s'élèvent seulement à 2—3 mètres au dessus du fond des étangs. Une végétation naturelle s'y trouve, peu changée sous l'influence de l'activité humaine. Les mas sont relativement rares, les vignobles occupent une surface minimale, encore plus restreinte depuis les dernières années. Les dunes de la Petite Camargue sont en contact immédiat sur une étendue considérable avec celles du cordon littoral, mais n'ont aucun contact avec des formations géologiques plus anciennes. Elles touchent uniquement sur un espace restreint aux plus anciennes dunes par l'intermédiaire de dunes situées au Sud d'Aiguesmortes. Elles sont d'ailleurs entourées de toutes parts par des sols salés, occupés par une végétation spécifique, tandis que par exemple des espèces des forêts du chêne vert n'ont pas de chance pour s'installer. Les dunes situées au Sud d'Aiguesmortes occupent, au point de vue de l'âge, une position intermédiaire entre les plus anciennes dunes de Silve Godesque et les dunes plus jeunes de la Petite Camargue. Elles sont plates et leur végétation primitive est presque complètement détruite, en partie à l'occasion de l'exploitation et le transport du sel (Salines du Midi) et aussi par des vignes nouvellement plantées. A peine çà et là se sont conservés des individus du groupement à *Artemisia campestris* var. *glutinosa*, ainsi que des touffes séparées de

Juniperus phoenicea et de *Phyllirea angustifolia*. Le Bois de Jarras est le seul fragment plus considérable de végétation moins dévastée.

Le sable de toutes ces dunes est très riche en carbonate de calcium (20—30%). L'analyse donne 80 et parfois 95% des particules de 1—0,1 millimètres de diamètre.

L'isolement des dunes étudiées éloignées des formations géologiques plus anciennes et le contenu en carbonate de calcium élevé du sable ont eu une influence décisive sur le développement de la végétation.

b. Les conditions climatiques

La contrée étudiée subit des vents violents et fréquents, surtout du NO ou NE et SE. Ils ont une grande influence sur la végétation et son développement. Il ne s'agit pas seulement de l'action physiologique du vent qui assèche, mais encore de la dissémination des graines, de son influence sur l'érosion des dunes en rendant plus difficile leur stabilisation. Les eaux des étangs et la mer adoucissent le climat. En général les hivers sont ici moins rigoureux et les chaleurs de l'été moins fortes qu'à l'intérieur du pays. Les gelées sont relativement rares et il neige rare-

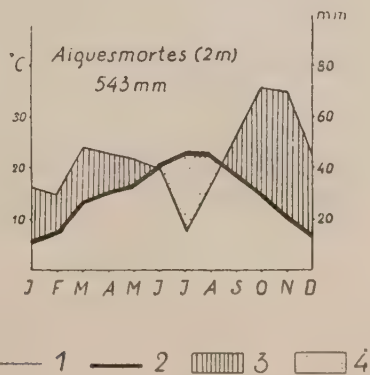


Fig. 2. Diagramme climatique d'Aiguesmortes: 1 — cours de précipitations mensuelles moyennes; 2 — cours de températures moyennes; 3 — saison humide; 4 — saison de sécheresse

ment. Les pluies sont violentes concentrées sur un petit nombre de jours (pour Aiguesmortes 52 en moyenne). Il pleut surtout en automne, en hiver et au commencement du printemps; les étés sont secs.

La répartition des précipitations et les cours de température mensuelle pour Aiguesmortes se trouve sur fig. 2 (selon les données de Galtier 1958). Le total annuel des pluies à Aiguesmortes est de 543 millimètres, aux Saintes-Maries de 504 millimètres.

VÉGÉTATION

a. Données floristiques *

Il semble intéressant de donner ici quelques indications des plantes rares ou peu connues observées par moi sur les dunes au Nord d'Aigues-mortes et en Petite Camargue.

1. *Galilea mucronata* (L.) Paul. (*Cyperus capitatus* Vand.) — au Nord d'Aiguesmortes très rare: Mas de Trouchaud, à l'Ouest de Caves du Grand Chaumont.

2. *Pancratium maritimum* L. — Mas de Trouchaud.

3. *Limodorum abortivum* L. — au Nord d'Aiguesmortes: Mas de l'Abbé, Mas du Garde.

4. *Cephalanthera rubra* (L.) Rich. — près du Mas du Petit Chaumont.

5. *Epipactis microphylla* (Ehrh.) Swartz. — *Quercetum galloprovinciale* appauvri près du Mas Grand St. Jean au Nord d'Aiguesmortes.

6. *Epipactis latifolia* (L.) All. — Mas de Trouchaud.

7. *Orchis fragrans* Poll. — à l'Ouest du Grau-du-Roi.

8. *Ophrys atrata* Lindl. — Petite Camargue, près de Maison le Ferradou.

9. *Euphorbia palustris* L. — Silve de Montcalm, dépression parmi les dunes.

10. *Cerastium balearicum* Hermann C. *dentatum* Möschl („Alles C. *semidecandrum* „L" aus dem Mediterrangebiet mit Ausnahme der Apenninen-Halbinsel mit Sizilien" — Möschl 1937) — fort répandue sur les dunes anciennes.

11. *Malcolmia confusa* Boiss. — Petite Camargue près de la mer au Sud de Caves de la Pinède; Mas de la Pinède; Radeaux des Deux Pins.

12. *Malcolmia littorea* (L.) R. Br. — fort répandue au Nord d'Aiguesmortes et en Petite Camargue.

13. *Rhamnus cathartica* L. — Mas de l'Abbé au Nord d'Aiguesmortes, exemplaires peu typiques.

14. *Onosma pyramidatum* Br.-Bl. — Mas de l'abbé et Mas de Trouchaud.

15. *Linaria cirrosa* (L.) Willd. — à l'Ouest du Grau-du-Roi, Silve de Montcalm et Petite Camargue près du Mas de la Pinède.

16. *Phelypaea arenaria* (Borkhaus) Walpers — Mas de l'Abbé.

17. *Phelypaea nana* Noë — Bois près du Mas Jarras; Petite Camargue près de Maison le Ferradou.

b. Groupements végétaux

Observées de loin les dunes anciennes donnent l'impression d'être entièrement couvertes de forêts de pins pignons. Cependant en y regardant de plus près on aperçoit que la végétation arborescente et arbustive est très disloquée et qu'elle forme une mosaïque.

Sur le terrain étudié furent constatés et brièvement caractérisés les groupements végétaux suivants, appartenant aux 4 ordres.

* La nomenclature des espèces d'après Fournier (1946) et Braun-Blanquet (1952).

- I. l'Ordre: *Ammophiletalia*
 - 1. *Ammophiletum arundinaceae* (fragm.)
 - 2. Groupement à *Artemisia campestris* var. *glutinosa*
- II. l'Ordre: *Thero-Brachypodietalia*
 - 3. *Staticetum echinoidis*
- III. l'Ordre: *Quercetalia ilicis*
 - 4. *Quercetum ilicis galloprovinciale* (appauvri)
 - 5. Groupement à *Juniperus phoenicea* var. *lycia* et *Phyllirea angustifolia* de l'alliance *Quercion ilicis*
- IV. l'Ordre: *Populetales albae*
 - 6. *Populetales albae*

1. *Ammophiletum arundinaceae*

Les associations de l'ordre *Ammophiletalia* ont leur optimum de développement dans les dunes récentes (Kühnholz-Lordat 1923, Braun-Blanquet 1952). Les dunes anciennes ne leur offrent plus de conditions favorables de développement. Certaines espèces caractéristiques des dunes s'y trouvent largement répandues, par exemple *Malcolmia littorea*, mais les associations typiques pour les dunes ne sont formées que fragmentairement. Des individus appauvris d'*Ammophiletum* se rencontrent dans les dunes de l'ancien cordon littoral près du Mas du Chaumont, où le vieux cordon littoral est lié aux dunes récentes et en Petite Camargue près de la Maison le Ferradou et du Grand Radeau. En Petite Camargue le nombre des caractéristiques de dunes s'agrandit successivement en s'approchant du bord de la mer. Près de la Maison le Ferradou on trouve déjà 10 espèces, entre autres: *Polygonum maritimum*, *Euphorbia paralias* et *Ammophila arenaria*.

L'*Ammophiletum* le mieux développé que j'ai trouvé dans les dunes étudiées est représenté par un relevé fait le 26 mai 1959 sur la crête haute de 6 mètres, située à l'Ouest des Caves du Grand Chaumont en bordure du vignoble. Recouvrement de la végétation 60%; surface du relevé 150 m².

Caractéristiques de l'association:

2.2—3 *Ammophila arenaria* (L.) Link ssp. *arundinacea* (Host).

Caractéristiques de l'alliance (*Ammophilion*) et de l'Ordre (*Ammophiletalia*):

- 1.2 *Malcolmia littorea* (L.) R. Br.
- +2 *Helichrysum stoechas* (L.) DC. var. *maritimum* (J. et F.) Rouy
- + *Pancratium maritimum* L.
- 2.2 *Teucrium polium* L. var. *maritimum* Alb. et Jah.
- 1.1 *Scleropoa maritima* Parl.
- 1.1 *Vulpia fasciculata* (Forsk.) Fritsch [*V. uniglumis* (Sol.) Dum.]
- +2 *Artemisia campestris* L. var. *glutinosa* Ten.
- + *Galilea mucronata* (L.) Parl. (*Cyperus capitatus* Vand.)
- + *Ononis ramosissima* Desf.
- + *Scrophularia canina* L. ssp. *humifusa* Gaut.
- + *Erianthus Ravennae* (L.) P. B.

Compagnes:

- + *Centaurea aspera* L.
- + *Corynephorus articulatus* (Desf.) P. B.
- + *Crepis taraxacifolia* Thuill.
- + *Ephedra distachya* L.
- + *Medicago minima* L.
- + *Oenothera biennis* L.
- + *Osyris alba* L.
- + *Phleum arenarium* L.
- + *Populus nigra* L. (juv.)
- + *Imperata cylindrica* (L.) P. B.

On remarque dans le relevé étudié le manque de la majorité des caractéristiques de l'association (en dehors d'*Ammophila arenaria*, qui domine). En revanche les espèces caractéristiques de l'alliance *Ammophilion* et de l'ordre *Ammophiletalia* sont représentées en plus grand nombre. Ces individus d'association, au moins en partie et indirectement, doivent leur existence à l'homme. La coupe des forêts voisines a influencé l'augmentation de l'érosion éolique qui empêche la stabilisation du sable et l'envahissement de la dune par la végétation forestière. Sur la même dune tout près de l'individu étudié, croissent cependant déjà *Quercus pubescens* et *Q. ilex*.

2. Groupement à *Artemisia campestris* var. *glutinosa*
(tab. 1) *

Sur les sables incomplètement stabilisés de Silve Godesque et de la Petite Camargue se développe un groupement, dans lequel dominent alternativement: *Artemisia campestris* var. *glutinosa*, *Helichrysum stoechas* var. *maritimum* ou *Teucrium polium* var. *maritimum*. Ces espèces croissent ordinairement sur le versant opposé au vent des dunes récentes; elles sont caractéristiques du *Crucianelletum*. *Rumex tingitanus* croît ici en abondance. Dans ce groupement se trouvent en outre des espèces caractéristiques de l'ordre des *Ammophiletalia* comme *Malcolmia littorea* et plus rarement *Pancratium maritimum*, *Galilea mucronata* et *Ammophila arenaria*. Le groupement subit moins l'action du vent que la végétation des dunes récentes. Parmi les touffes d'*Artemisia campestris* croissent des espèces propres aux pelouses xerothermiques calcaires de l'alliance du *Thero-Brachypodion*: *Arenaria serpyllifolia* ssp. *leptoclados*, *Medicago minima* et *Clypeola microcarpa*.

Molinier et coll. (1959) ont publié des relevés à *Artemisia campestris* var. *glutinosa* qui domine dans les dunes anciennes à Beynes, à quelques kilomètres au Sud d'Arles. Ils ont décrit une nouvelle associa-

* Tous les tableaux appartenant à l'article de Mr Zarzycki sont placés à la fin du livre.

tion *Artemisieta-Holoschoenetum romani* Mol. et Tallon et la placent dans l'alliance du *Holoschoenion romani* Mol. et Tallon 1959. Les relevés de la Petite Camargue et de Silve Godesque se rattachent à ceux de Beynes quoiqu'un *Rumex tingitanus* ne s'y trouve pas et que les espèces de l'ordre *Thero-Brachypodietalia* (13 espèces) y sont plus fortement représentées tandis que les plantes dunales diminuent.

Le groupement à *Artemisia campestris* var. *glutinosa* est peu homogène, mais son caractère dynamique est nettement accusé; il constitue un stade de succession suivant le *Crucianelletum*. Dans des conditions favorables ce groupement est suivi par des fourrés à *Phillyrea angustifolia* et *Juniperus phoenicea* var. *lycia*, pour se transformer successivement en forêt, où *Quercus pubescens* est fortement représenté.

3. *Staticetum echiodis* (tab. 2)

Le *Staticetum echiodis* (Tallon) Braun-Blanquet 1931 se développe en Petite Camargue dans des endroits plats ou légèrement inclinés, abrités contre l'action du vent par des touffes du *Juniperus phoenicea* ou par *Phillyrea angustifolia*. Le sable est toujours assez stable, riche en carbonate de calcium, avec des traces de sel. Le tapis végétal composé surtout de therophytes couvre 30 à 90% du sol (le plus souvent ca. 60%). Les espèces vivaces y sont peu nombreuses. C'est une association naturelle, contrairement à d'autres groupements végétaux de l'alliance du *Thero-Brachypodion*. Le développement annuel du *Staticetum echiodis* commence très tôt (au mois de mars) par la floraison du *Myosotis collina*. Le plein épanouissement se fait au mois d'avril et de mai. Au début de juin déjà la majorité des espèces se dessèchent et au mois de juillet tout est desséché.

Plusieurs espèces peuvent être considérées comme caractéristiques de l'association, mais en grande partie seulement comme caractéristiques locales: *Statice echiodis*, *Avellinia Michellii*, *Catapodium loliaceum*, *Malcolmia confusa* (car. loc.), *Linaria cirrosa* (car. loc.) et *Viola nemausensis* (car. loc.). L'appartenance de l'association à l'alliance *Thero-Brachypodion* et à l'ordre *Thero-Brachypodietalia* est hors de doute. Elle est prouvée par un nombre des caractéristiques (*Cerastium balearicum*, *Vaillantia muralis*, *Crepis bulbosa*, *Ononis reclinata*, *Saxifraga tridactylites*, *Clypeola microcarpa*, *Arenaria serpyllifolia* ssp. *leptoclados*, *Scleropoa rigida* et beaucoup d'autres). Par contre, je n'ai pas réussi à trouver en Petite Camargue *Evax pygmaea*, *Bupleurum semicompositum* et *Myosotis pusilla*, indiquées par Braun-Blanquet (1952) comme caractéristiques (d'ailleurs assez rares).

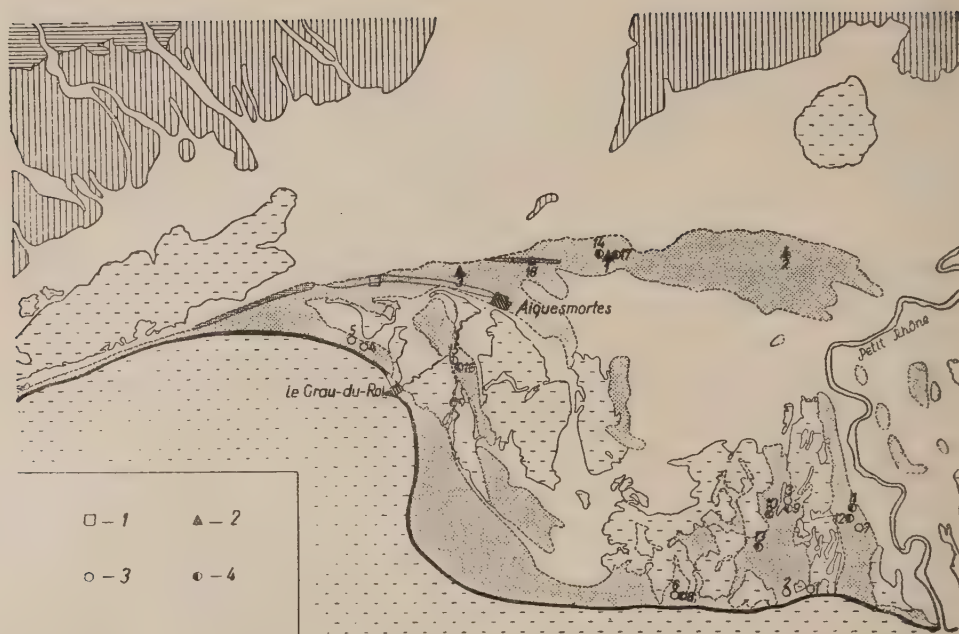


Fig. 3. Répartition des relevés phytosociologiques: 1 — *Ammophiletum arundinaceae* (texte, p. 583); 2 — groupement à *Artemisia campestris* var. *glutinosa* (tab. 1); 3 — *Staticetum echiioidis* variante typique (tab. 2); 4 — *Staticetum echiioidis* variante à *Vulpia longiseta* (tab. 2)

Bon nombre d'espèces caractéristiques des pelouses méditerranéennes calcaires sont rares dans les dunes anciennes, par exemple *Parentuccella latifolia*, *Sideritis romana*, *Campanula erinus*, *Helianthemum salicifolium*, *Scabiosa maritima* et *Minuartia mediterranea*. Le *Staticetum echiioidis* est déjà bien développé sur une surface de 10 à 30 m². Le nombre des plantes vasculaires dans les individus particuliers oscille entre 12 et 43. Les mousses et les lichens manquent dans plusieurs relevés, dans d'autres ils recouvrent jusqu'à 70% de la surface du sol. Parmi les mousses se trouve le plus souvent *Trichostomum flavovirens*, rare ailleurs. *Cladonia endiviaefolia* est assez fréquent; moins souvent on rencontre *Cladonia rangiformis*. Le *Staticetum echiioidis* est plus répandu en Petite Camargue que dans les dunes anciennes à Silve Godesque, plus ombragées. Les individus provenant d'ici sont plus pauvres en espèces (relevés no 14—18), quoiqu'ils appartiennent encore sans doute à cette association. Une variante appauvrie dominée par *Vulpia longiseta* se développe surtout dans les forêts clairiérées de pin pignon, à l'ombre pendant une partie de la journée.

La présence de semis de *Phillyrea angustifolia*, *Cistus salviifolius*, *Pinus pinea* et *Rosmarinus officinalis* dans le *Staticetum* prouve que

la succession naturelle tend vers le fourré à *Phillyrea angustifolia* ou vers la forêt à *Pinus pinea*.

4. Groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia*
(de l'alliance *Quercion ilicis*)
(tab. 4a)

Les groupements arbustifs de la Camargue ont été caractérisés brièvement par M. Tallon en 1931. Il en a donné une caractéristique floristique et écologique et a tracé le développement de deux unités phyto-

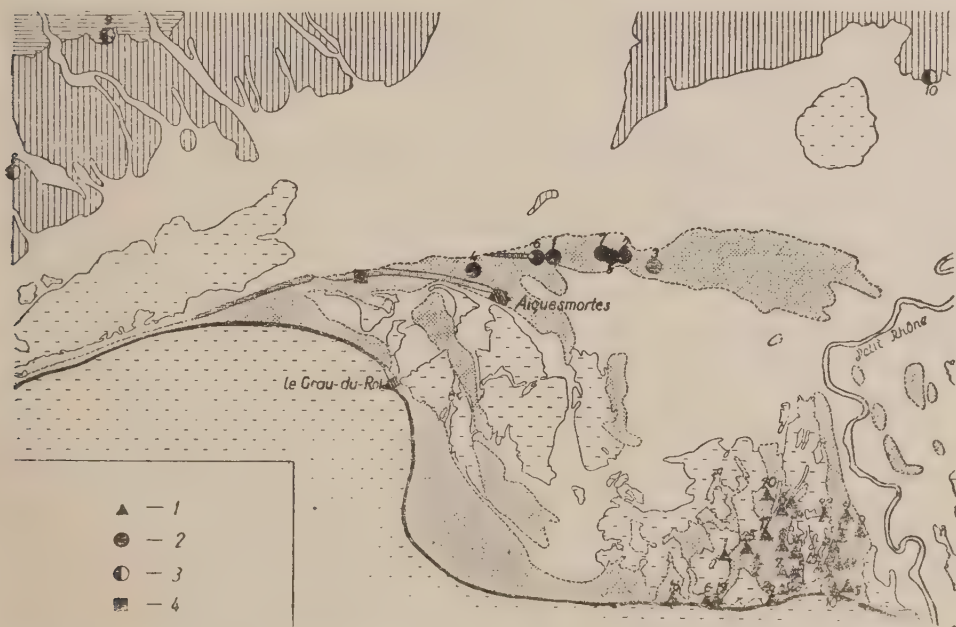


Fig. 4. Répartition des relevés phytosociologiques: 1 — groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia* (tab. 4a); 2 — *Quercetum galloprovinciale* (dunal, appauvri) (tab. 5); 3 — *Quercetum galloprovinciale pubescentetosum* (tab. 5); 4 — *Populetum albae* (texte, p. 594)

sociologiques: l'association à *Phillyrea angustifolia* et *Jasminum fruticans* de la partie centrale de la Camargue et la forêt à *Juniperus phoenicea* des Rièges sans cependant publier de tableaux phytosociologiques complets.

Braun-Blanquet (1952), en s'appuyant sur trois relevés du bois des Rièges (faits avec M. Tallon) a rattaché le groupement à *Juniperus phoenicea* à l'association du chêne vert comme sous-association distincte: *Quercetum galloprovinciale juniperetosum*. Molinier et coll. (1959) présentent un *Phillyreetum* sur les dunes situées au Sud

d'Arles. Molinier a décrit aussi de la Provence une sous-association à *Juniperus phoenicea* var. *lycia* de l'Oleo-Lentiscetum provinciale, que l'on retrouve entre autres en Sardaigne. Des junipéraies analogues (groupement à *Juniperus lycia*) de l'Oleo-Ceratonion ne sont pas rares à Majorque où elles prennent parfois une extension considérable (Bolos et Molinier 1960). En Catalogne continentale, des peuplements semblables ont été observés par Bolos. Ils font la transition entre le complexe *Ammophilion* — *Critmo-Statition* d'une part et le *Querceto-Lentiscetum* d'autre part.

Les matériaux assemblés par moi en Petite Camargue indiquent que les individus avec dominance du *Phillyrea angustifolia* ou du *Juniperus phoenicea* var. *lycia* ou enfin des deux espèces ensemble ne représentent pas une unité phytosociologique homogène ni au point de vue floristique ni par leur évolution.

Les relevés réunis dans le tableau 3 représentent le groupement le plus typique, le moins modifiés par l'action de l'homme. Ils sont largement répandus en Petite Camargue et aux Rièges. Notre tableau comprend 20 relevés de la Petite Camargue, dans lesquels dominent *Phillyrea angustifolia* ou *Juniperus phoenicea* et 7 relevés différant par la dominance du pin parasol. On a ajouté 5 relevés du Bois des Rièges, dont 2 publiés déjà par Tallon (1955) et 3 de Braun-Blanquet et Tallon pas encore publiés*.

Le tableau 3 indique, que dans les dunes de la Petite Camargue et des Rièges se développe seulement un groupement, dans lequel dominent tour à tour *Phillyrea angustifolia* et *Juniperus phoenicea*; ils croissent parfois ensemble. Dans certains relevés apparaît *Pinus pinea*. Pour la plupart ce sont des fourrés hauts de 4—5 mètres, rarement de 6 mètres. Le genévrier rouge atteint au maximum 10 mètres aux Rièges. Ces buissons à genévriers ne couvrent pas les dunes d'une façon continue; ils forment des fourrés semblables à des îlots parfois assez étendus et presque impossibles à traverser à cause de nombreuses plantes grimpantes comme *Rubia peregrina*, *Asparagus acutifolius*, *Smilax aspera* etc. Certains peuplements sont traversés par des sentiers frayés par les taureaux demi-sauvages et par des clairières envahies généralement par le *Staticeum echiodis*. Les espèces herbacées croissent d'habitude sur les bords, moins souvent à l'intérieur des buissons. Dans ce groupement existent en abondance des caractéristiques de l'ordre des *Quercetalia ilicis* (*Phillyrea angustifolia*, *Pistacia lentiscus*, *Daphne gnidium*, *Rubia peregrina*, *Smilax aspera*, *Clematis flammula* ssp. *flammula*, *Rhamnus alaternus*).

* Je remercie M. J. Braun-Blanquet qui a eu l'obligeance de mettre à ma disposition ces relevés.

Les caractéristiques de l'alliance du *Quercion ilicis* sont moins nombreuses (*Asparagus acutifolius*, très rarement *Vincetoxicum nigrum*). Les caractéristiques de l'association du *Quercetum galloprovinciale* manquent presque totalement; sauf *Phillyrea media*, en général rare en Camargue (Tallon 1930), qui est présente dans 13% des relevés. *Ruscus aculeatus* et *Lonicera implexa* sont encore plus rares. Ces circonstances ne permettent pas, à notre avis, de considérer la junipéraie dunale de la Petite Camargue comme une sous-association du *Quercetum galloprovinciale*.

On ne peut douter que le groupement en question appartient à l'ordre *Quercetalia ilicis* et à l'alliance *Quercion ilicis*. La question se pose, s'il ne pourrait pas être considéré comme une association à part. Cependant celle-ci manquerait des bonnes caractéristiques. Même *Juniperus phoenicea* ne peut être reconnu comme espèce caractéristique, à cause de son abondance dans différents groupements du Languedoc et de Provence. Il croît surtout dans les fissures des roches calcaires et parfois même en grande abondance dans le *Rosmarineto-Lithospermetum*. La var. *lycia* du *Juniperus phoenicea* est limitée aux dunes et diffère de la forme typique par son port et par la dimension de ses semences, mais la valeur systématique de ce taxon est faible*. En outre il apparaît en Provence, dans les Baléares et au Maroc dans les groupements de l'*Oleo-Ceratonion*.

Provisoirement nous déterminons notre unité végétale de la Petite Camargue comme le groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia* de l'alliance *Quercion ilicis* en évolution vers le *Quercetum galloprovinciale*.

Dans ce groupement sont fréquents: *Cistus salviifolius*, *Rosmarinus officinalis*, *Crepis bulbosa*, *Holoschoenus vulgaris* ssp. *romanus*, *Teucrium polium* var. *maritimum* et *Asphodelus cerasifer*. Certaines espèces (par exemple: *Anthriscus vulgaris*, *Sonchus oleraceus*, *Stellaria media*) ont été apportées ici par des animaux paissants. Parmi les lichens uniquement *Cladonia endiviaefolia* est rencontrée plus fréquemment et parmi les mousses *Trichostomum (Tortella) flavovirens*.

On peut distinguer dans notre groupement quelques faciès plus ou moins remarquables: à *Juniperus phoenicea*, à *Phillyrea angustifolia*, à *Pistacia lentiscus*, à *Rosmarinus officinalis* et une variante à *Pinus pinea*. Cette dernière se distingue singulièrement surtout s'il s'agit de la

* Sur les dunes de la Petite Camargue et aussi aux Rièges (Tallon 1931) on trouve des exemplaires du *Juniperus phoenicea* var. *lycia* qui sont peu typiques. D'après Molinier et coll. (1959) „l'étude du genévrier rouge, effectuée sur la morphologie, l'anatomie, la chimie, n'a pas permis de distinguer le genévrier du littoral de celui des montagnes dont les peuplements sont absolument distincts géographiquement et floristiquement”.

physionomie. Ce sont en principe déjà des bois de pin parasol, qui atteignent la hauteur de 10 à 12 mètres. La strate arborescente se compose des mêmes espèces qui se développent en dehors de ces bois, dans lesquels leur vitalité est sensiblement réduite. Uniquement *Crepis bulbosa* se développe mieux qu'ailleurs dans l'ombre des pins. *Juniperus phoenicea* domine dans les individus exposés à l'action des vents plus forts, situés auprès de la mer et aux bords de petites îles dispersées parmi les étangs. À mesure qu'on s'éloigne du bord de la mer le rôle du genévrier diminue, il devient plus solitaire. Il est très rare dans les dunes au Nord-Ouest d'Aigues-mortes et assez fréquent au Sud de cette ville. En Petite Camargue, de même qu'aux Rièges, le genévrier se régénère faiblement. Les jeunes exemplaires sont très rares. Une seule fois dans le relevé no 11 (tab. 4a) sur une surface de 100 m² j'ai réussi à compter 19 jeunes exemplaires hauts de 10 à 20 centimètres. Ils se trouvent le plus souvent au bord des buissons serrés.

Tableau 3

Moyens de dissémination dans trois groupements apparentés: *Quercetum ilicis galloprovinciale*, *Quercetum ilicis galloprovinciale* des dunes anciennes (appauvri) et groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia*

Le type de la dissémination	<i>Quercetum ilicis galloprovinciale</i> (Br. — Bl. 1936)		<i>Quercetum ilicis galloprovinciale</i> des dunes anciennes (appauvri)		Groupement à <i>Juniperus phoenicea</i> et <i>Phillyrea angustifolia</i>	
	nombre d'espèces	%	nombre d'espèces	%	nombre d'espèces	%
Endozoochores	24	43,7	15	40,6	13	28,9
Myrmécochores	6	10,9	0	0,0	1	2,2
Barochores	7	12,7	2	5,4	2	4,4
Anémochores	11	20,0	9	24,3	18	40,0
Ombrohydrochores	0	0	0	0	2	4,4
Epizoochores	0	0	4	10,8	1	2,2
Dyszoochores	3	5,4	4	10,8	2	4,4
Autochores	4	7,3	3	8,1	6	13,5
Nombre d'espèces	55	100,0	37	100,0	45	100,0

Les endroits moins exposés à l'action du vent sont envahis par le maquis à *Phillyrea angustifolia*. Cette espèce parvient jusqu'au bord de la mer, mais des peuplements compacts ne s'y rencontrent que dans les dépressions parmi les dunes. *Rosmarinus officinalis* se développe en masse sur des terrains récemment incendiés.

Le groupement plus ou moins semblable réapparaît aux Rièges, cependant le rôle de *Phillyrea angustifolia* y est moins important. De même qu'en Petite Camargue on peut distinguer au Bois de Rièges un faciès à *Pistacia lentiscus* (à l'Est), et un autre à *Rosmarinus* (à l'Ouest). Mais on n'y rencontre pas un seul individu de *Pinus pinea* (Tallon 1931).

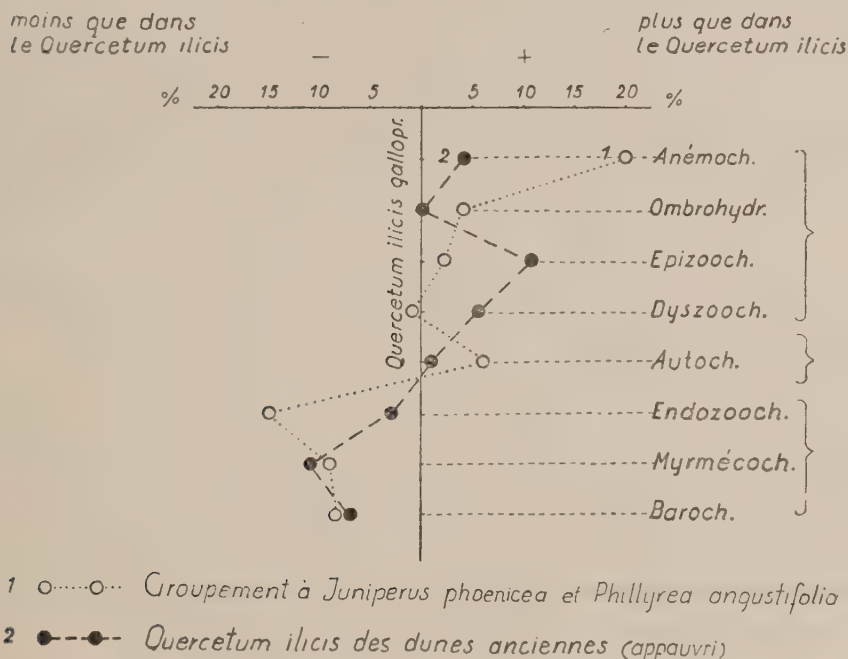


Fig. 5. Graphique comparative* de dissémination dans trois groupements apparentés : *Quercetum ilicis galloprovinciale*, *Quercetum ilicis galloprovinciale* dunal et groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia*

* Ce diagramme fut exécuté sur le modèle de la méthode graphique de Jentys-Szaferowa (1959). La ligne verticale représente la composition du *Quercetum ilicis galloprovinciale* servant d'unité de comparaison. Les types biologiques comparés furent énumérés du côté droit du diagramme; dans le cas étudié le pourcent de participation des types de la dissémination. Si dans l'unité comparée (par exemple dans le groupement à *Juniperus phoenicea* et *Phillyrea angustifolia*) le pourcent de participation d'un type se trouvait supérieur à celui du *Quercetum galloprovinciale* (v. tab. 5) — on marquait la différence à droite de la ligne verticale, s'il était inférieur — à gauche. Par exemple: dans le *Quercetum* le pourcent des anémochores est de 20%, dans le groupement à *Juniperus phoenicea* et *Phillyrea angustifolia* de 40%. Il y a 20% de plus d'anémochores dans ce deuxième groupement que dans l'unité comparative et cela fut marqué par un point dans le diagramme à droite de la ligne verticale.

Comme l'a souligné M. Tallon, le groupement à *Phillyrea angustifolia* de la Camargue est caractérisé par la présence d'un grand nombre d'espèces endozoochores (ornithochores). En comparant à cet égard le

groupement à *Juniperus phoenicea* et *Phillyrea angustifolia*, le *Quercetum galloprovinciale* (appauvri) de dunes anciennes et le *Quercetum galloprovinciale typicum* on remarque des différences considérables (tab. 3, fig. 5). Dans le groupement à genévrier rouge le pourcent des anémochores est frappant. Il y a aussi plus de dyszoochores, d'ombrohydrochores et d'épizoochores que dans l'association climax et moins d'endozoochores, de myrmécochores et de barochores. D'après M^{me} Molinier et Müller (1938) les endozoochores, les myrmécochores et les barochores sont de plus grande importance dans l'association climax, les anémochores, les ombrohydrochores et les épizoochores dans les stades de la succession précédant l'association climatique. Le grand nombre d'épizoochores dans le *Quercetum galloprovinciale* dunal s'explique par le voisinage d'enclos pour les animaux qui visitent continuellement les forêts dans les dunes.

Un groupement différent se trouve aux extrémités orientale et occidentale des dunes de la Silve Godesque, sur les dunes plates et basses. On peut y discerner deux types dépendant de l'élévation au-dessus du niveau de l'eau phréatique.

Sur des petites élévations dans le maquis à *Phillyrea angustifolia* dont la couverture arbustive dépasse parfois 70—80%, se trouvent des chênes, surtout *Quercus pubescens*, et les caractéristiques de l'ordre des *Quercetalia ilicis* (*Ruscus aculeatus*). Ces peuplements sont pour la plupart naturels, plus rarement dus à l'action de l'homme, qui abat les chênes en favorisant de cette manière les buissons. On a classé ces fourrés comme *Quercetum galloprovinciale* (appauvri) (tab. 5, relevé no. 4). Des maquis du même caractère se trouvent également dans les dunes anciennes à Beynes, à quelques kilomètres au Sud d'Arles (Molinier et coll. 1959).

Cependant certains peuplements dominés par le *Phillyrea angustifolia* (tab. 4b) semblent s'être développés après la coupe des forêts riveraines; leur régénération tend nettement vers le *Populetum albae*. Je cite comme exemple deux relevés faits dans des dépressions entre les dunes de la Silve de Montcaim. Ils diffèrent de ceux de la Petite Camargue par le manque du genévrier rouge et par la présence de *Populus alba*, *Ulmus campestris* et d'autres plantes, qui indiquent une plus grande humidité du sol (*Schoenus nigricans*, *Carex glauca*, *Carex distans*, *Arum italicum*). Les espèces de l'ordre *Quercetalia ilicis* (par ex. *Lonicera etrusca*, *Daphnognidium*, *Rhamnus infectoria* etc.) s'y trouvent également. Les fourrés qui se développent en bordure des dépressions entre les dunes périssent parfois en masse, probablement parce que leur racines sont périodiquement inondées.

5. Le *Quercetum ilicis galloprovinciale* (dunal, appauvri)
(tab. 5)

Le schéma de la succession végétale dans les dunes riches en carbonate de calcium (série de l'*Ammophilion*) a été donné par Braun-Blanquet (1936). Le stade terminal de la succession naturelle (progressive) de cette végétation est représenté par le *Quercetum galloprovinciale* (climax). Un relevé du *Quercetum galloprovinciale* (faciès à *Juniperus macrocarpa*) fait par Braun-Blanquet (l.c.) sur les dunes à 100 mètres de la mer près de San Vincenzo (Toscane) paraît confirmer cette supposition. Le stade terminal de la succession est formé par un maquis de *Quercus ilex* très dense, tondu par le vent, relativement pauvre en espèces, haut de 3 à 4 mètres, qui s'avance jusqu'à la plage. Outre le chêne vert s'y trouvent maintes espèces, caractéristiques de l'association ou des unités phytosociologiques supérieures (*Phillyrea media*, *Lonicera implexa*, *Ruscus aculeatus*, *Viburnum tinus*, *Pistacia lentiscus* etc.). „Cette forêt de Chêne vert sur les dunes à quelques mètres du bord de la mer est une preuve irréfutable de la possibilité d'installation du *Quercetum ilicis* comme climax à l'emplacement de l'*Ammophilion* pionnier" (Braun-Blanquet l.c.). Molinier et Tallon (1949) arrivent à la même conclusion en ce qui concerne la Crau et la Camargue.

Dans les dunes de l'ancien cordon littoral (Silve Godesque) des individus d'association du *Quercetum ilicis galloprovinciale* se développent également mais ils sont fragmentaires et appauvris par rapport aux forêts de Chêne vert de l'intérieur. Ce ne sont que des précurseurs isolés de l'association. Ces bois occupent des terrains peu étendus, situés le plus souvent dans le voisinage d'habitations. Leur physionomie est nettement différente du *Quercetum galloprovinciale* typique car l'arbre dominant est le pin parasol (*Pinus pinea*). Celui-ci arrive parfois à la hauteur considérable de 20 mètres et même plus et atteint un diamètre de 60 (70) centimètres. Le degré de recouvrement varie entre 30 et 90%, en moyenne 60—70. Le sous-bois est généralement formé par les chênes, surtout par *Quercus pubescens*. Parfois les chênes dominant et atteignent la hauteur de 18 mètres. Parmi les espèces caractéristiques du *Quercetum galloprovinciale* apparaissent seulement *Ruscus aculeatus* en abondance et *Epipactis microphylla* sporadiquement ainsi que *Viburnum tinus* (spontané?). Les caractéristiques de l'alliance de *Quercion ilicis*, outre *Quercus ilex* lui-même, sont représentées par *Asparagus acutifolius* et *Lonicera etrusca*; les caractéristiques de l'ordre par *Clematis flammula* ssp. *flammula*, *Phillyrea angustifolia*, *Rhamnus alaternus* et *Rubia peregrina*. Rares sont *Quercus coccifera* et *Daphne gnidium*. Le développement des buissons est luxuriant. Le recouvrement de la strate herbacée est médiocre (5 à 60%). Les mousses manquent presque complètement.

Pour démontrer la pauvreté spécifique des peuplements situés dans les dunes anciennes trois relevés du tab. 5 (relevés no 8—10) ont été faits par moi dans les formations pliocènes du voisinage. On constate dans les dunes l'absence de nombreuses caractéristiques de l'association. Par contre, on trouve des espèces plus ou moins nitrophiles ou à demi-synantropiques (*Centaurea aspera*, *Anthriscus vulgaris*, *Erigeron canadensis*, *Galium aparine*, *Sonchus oleraceus* et d'autres) amenées ici par des animaux ou l'homme. Notre peuplement se rapproche le plus de la sous-association *pubescentetosum* du *Quercetum galloprovinciale* (Kielhauser 1939, Braun-Blanquet 1952); cependant les espèces différentielles de cette sous-association y sont représentées uniquement par le *Quercus pubescens*, qui s'y trouve en grande abondance. C'est probablement en rapport avec une humidité plus élevée du sol et de l'air due au niveau relativement haut de l'eau phréatique*.

Le groupement en question est le mieux développé aux endroits suivants: Mas de Trouchaud, Mas de l'Abbé et Mas Grand St. Jean. À l'Ouest du Mas de Trouchaud et à l'Est du Mas Grand St. Jean où les dunes deviennent plus basses, les peuplements sont plus pauvres, plus fragmentaires. L'importance des chênes diminue nettement. Par contre *Phillyrea angustifolia* se développe mieux. N'ayant plus de concurrence des arbres qui ne résistent pas à l'inondation périodique des racines, cette espèce forme d'abondants fourrés parmi lesquels croissent, ça et là quelques chênes peu nombreux.

6. *Populetum albae*

Le *Populetum albae* du Languedoc a été très bien décrit par Tchou (1948). Dans des dépressions parmi les plus anciennes dunes à Silve de Montcalm, près du Mas de l'Abbé et du Mas de Chaumont se trouvent des fragments de ce groupement riverain appartenant à l'ordre des *Populetalia albae*. Parmi les arbres domine généralement *Ulmus campestris*, parfois aussi *Populus alba* ou *Fraxinus oxycarpa*.

Le meilleur relevé de l'association vu par moi se trouve un peu plus vers l'Ouest du Mas de Chaumont. *Populus alba* y atteint 18—20 mètres, il a un diamètre de 40—60 centimètres. Le recouvrement des arbres 70%, des arbustes 90%, de la strate herbacée — 80%. Surface du relevé pris le 25 mai 1958 — 200 m²

Caractéristiques régionales de l'association:

2.1 *Fraxinus oxycarpa* Willd.

2.1 *Ulmus campestris* L.

4.2 *Populus alba* L.

* J'ai observé de l'eau le 2 mai 1959 dans la profondeur de 2,5 mètres (un puits situé dans l'individu d'association près du Mas de l'Abbé).

2.1 *Iris foetidissima* L.+ *Torilis arvensis* (Huds.) LinkCaractéristiques de l'ordre (*Populetales albae*):4.3 *Brachypodium silvaticum* (Huds.) P. B.Caractéristiques de la classe (*Querceto-Fagetea*):+ *Clematis vitalba* L.2.2 *Crataegus monogyna* Jacq.+ *Ligustrum vulgare* L.+ *Prunus spinosa* L.+ *Rosa canina* L.Espèces du *Quercetum ilicis galloprovinciale*:+ *Clematis flammula* L. ssp. *flammula* auct.+ 2 *Lonicera etrusca* Santi1.2 *Rubia peregrina* L.1.2 *Ruscus aculeatus* L.

Compagnes:

1.1 *Aristolochia clematitis* L.+ *Aristolochia rotunda* L.+ *Arctium minus* L.+ *Arum italicum* Mill.+ 2 *Brachypodium phoenicoides* R. et S.+ *Bromus sterilis* L.+ *Galium aparine* L.2.2 *Hedera helix* L.+ *Melandryum album* (Mill.) Garcke+ *Osyris alba* L.2.1 *Poa trivialis* L.3.3 *Rubus ulmifolius* Schott+ *Sonchus oleraceus* L.

7. Les sols des groupements végétaux étudiés

(tab. 6; fig. 6)

Un sable siliceux à gros grain est le substratum des sols formés sur les dunes. Ce sable est constitué par des particules de 0,1—1 mm de 80 à 95%. La teneur en carbonate de calcium de provenance organique (des débris de coquilles de mollusques) est généralement très grande (20—30%). Au cours du développement qui dépend strictement du cours de la succession de la végétation dans les dunes, a lieu une lente stabilisation de sable, qu'accompagne un accroissement d'acidification des horizons supérieurs des sols.

Les sols formés, du moment où ils sont cultivés, sont (Bordas 1944) des sols minéraux, sablonneux, calcaires et privés complètement de complexe absorbant argulo-humique. Ces sols sont généralement pauvres en azote, phosphore et potasse (1,5 p. mille pour N, de 25 mmg pour P₂O₅

Tableau 6

Analyses des sols

Groupement, localité	Profondeur cm	% des particules de grosseur (mm):							CaCO ₃ %	pH en		Perte en chauffant à ca 400° C	L'humus
		1—0,1	0,1— 0,05	0,05— 0,02	0,02— 0,006	0,006— 0,002	<0,002	H ₂ O		KCl			
1. Petite Camargue	0—2	89	2	3	2	1	3	23,0	7,5	7,5	1,61	.	
	10	89	3	1	1	3	3	23,0	7,7	7,6	1,42	.	
	30	88	4	1	3	1	3	24,0	7,8	7,7	1,10	.	
2. Mas de l'Abbé	0—4	85	5	2	3	1	4	17,5	7,5	7,3	.	.	
	15—20	90	3	1	3	0	3	19,5	7,6	7,4	.	.	
3. Bois à <i>Pinus pinea</i> Petite Camargue	0—5	.	.	.	.	.	.	15,8	6,7	6,4	21,81	12,62	
	7—10	91	1	3	1	0	4	22,5	7,7	7,4	1,58	0,65	
	25—30	95	2	3	1	1	2	23,0	7,7	7,6	0,97	.	
	45—50	95	1	1	0	1	2	24,5	8,1	7,9	0,59	.	
	5—10	.	.	.	.	.	.	5,6	6,6	5,8	58,88	37,62	
4. Petite Camargue	20	84	6	2	3	0	4	25,5	7,7	7,4	0,94	.	
	50	94	1	1	1	1	2	27,5	8,0	7,8	1,46	.	
5. Mas de l'Abbé	2—5	81	8	2	3	2	4	14,5	7,1	6,8	12,41	8,04	
	20—30	91	4	1	0	1	3	22,5	7,4	7,3	.	.	
6. Grand St. Jean	3—5	.	.	.	.	.	.	7,0	6,9	.	30,13	19,09	
	10—15	95	1	0	1	2	1	18,3	7,6	7,6	1,21	.	
7. Mas de Trouchaud	5	.	.	.	.	.	.	16,5	7,1	6,9	.	.	
	20	.	.	.	.	.	.	17,0	7,6	7,3	.	.	
	35—40	.	..	.	.	.	.	20,5	7,8	7,5	.	.	
8. Mas de Trouchaud	5	.	..	.	.	.	.	8,7	.	.	.	.	
	20	.	.	.	.	.	.	20,0	.	.	.	.	

et de 80 mmg pour K_2O — chiffres au-dessous desquels un rapport de ces éléments fertilisants est nécessaire). Ils sont aussi très perméables et le niveau de la nappe phréatique est assez bas.

Les sables mobiles des dunes récentes riches en calcaire sont occupés par l'*Ammophiletum*. Sur les dunes anciennes, sur le sable plus stabilisé, le groupement à *Artemisia campestris* var. *glutinosa* se développe. Le sable contient ici ca 20% de $CaCO_3$ et l'acidité oscille de 7,3 à 7,7 pH.

Le *Staticetum echiodis* occupe les sables déjà fortement stabilisés, dans des endroit abrités de vents violents. Le sable ne contient plus que des traces du sel. Il est encore difficile de différencier le profil du sol. Une couche superficielle de sable de 2 à 5 centimètres est seule légèrement plus foncée, ce qui indique qu'elle contient un peu de matière orga-

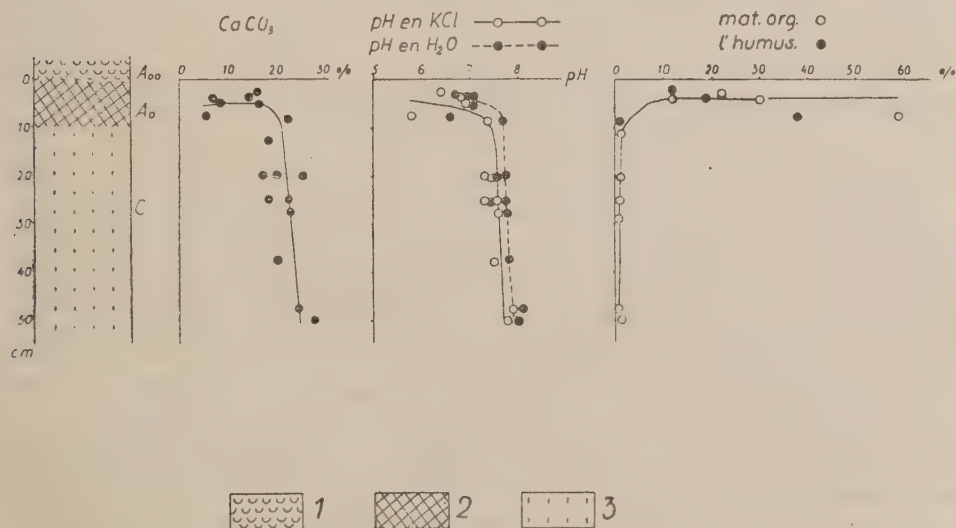


Fig. 6. Profil typique de sol, teneur en: $CaCO_3$, matière organique, l'humus et pH des sols dans les bois à *Pinus pinea*: 1 — litière non décomposé de *Pinus pinea*; 2 — litière peu décomposé avec addition du sable; 3 — sable riche en carbonate de calcium

nique. Le sable est aussi riche en $CaCO_3$ et cette teneur subit peu d'oscillations par rapport à la profondeur: 1 à 2% à la surface de moins que 10 ou 20 cm plus bas. De même l'acidité ne change pas depuis la surface jusqu'à 30—50 cm de profondeur étant de 7,5 à 7,8 pH. Il n'y a aucune différence entre l'acidité active (mesuré en H_2O) et échangeable (en KCl), qu'indique le manque d'ions d'hydrogène dans un complexe absorbant entièrement saturé de calcium.

Les sols du groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea* sont également peu développés. Dans les bois à *Pinus pinea* on a étudié 5 profils qui se ressemblent beaucoup.

Sous la couche de 2 (5) centimètres de feuilles sèches, non décomposées de *Pinus pinea* existe un horizon (de 15 à 30 cm) composé de feuilles émiettées, un peu collées, de couleur brune, mélangées de sable apporté par le vent. La teneur en matière organique oscille entre 12 et 59% et de l'humus entre 8 et 37%. Il contient toujours du carbonate de calcium qui varie entre 5 à 16%. Cet horizon est très légèrement acide ou neutre (pH en H₂O de 6,6 à 7,1; pH en KCl de 5,8 à 6,9). La limite entre l'horizon humifère et l'horizon minéral est fort accusé. A 20 (30) cm apparaît généralement un sable grossier basique contenant 20% et plus de carbonate de calcium (pH de 7,4 à 7,7).

Les sols des petites forêts claires et des buissons dunals sont exposées à la forte action de l'érosion éolienne, ce qui empêche la formation et la maturation du sol.

8. Quelques remarques sur le rôle de *Pinus pinea* dans le développement de la végétation des dunes

Dans les dunes de la Silve Godesque ainsi qu'en Petite Camargue le pin parasol, très répandu, montre une bonne vitalité. Il n'est cependant pas clair s'il y est spontané ou si après avoir été introduit par l'homme il s'est largement disséminé plus tard. Kühnholz-Lordat (1923) le considère comme espèce indigène dans les dunes de la Camargue. Braun-Blanquet (pendant une discussion) a des restrictions sérieuses à ce sujet. En Provence *Pinus pinea* est sans nul doute spontané, mais il y croît exclusivement sur le sol siliceux (Molinier et coll. 1959), tandis qu'il trouve dans les dunes du territoire étudié ici un sol riche en carbonate de calcium et il se régénère faiblement. Cet arbre est cultivé dans les pays méditerranéens depuis les temps romains (Fournier 1946) et il croît actuellement auprès de la plupart des mas de Camargue. Le manque de *Pinus pinea* aux Rièges surprend. S'il est indigène dans les dunes de la Petite Camargue pourquoi ne se trouve-t-il pas dans ce coin, ou l'homme n'a pas pu l'introduire?

Sur la carte du Diocèse de Nîmes (1720—1740) appartenant à SIGMA un petit bois, probablement de *Pinus pinea*, est marqué tout près d'Aiguesmortes, mais pas en Petite Camargue. Par contre, sur une carte de 1800 des bois de pin pignon figuraient déjà près du Mas de l'Abbé et en Petite Camargue.

Sans toucher la question de sa provenance spontanée ou subspontanée nous désirons seulement étudier l'influence de cette espèce sur le développement de la végétation. Kühnholz-Lordat (l.c.) s'est déjà occupé de cette question. Cette influence se manifeste de trois façons: par le freinage de la vitesse du vent, par l'ombrage et par la litière. Dans

l'ensemble l'influence n'est pas si grande qu'on pourrait supposer. Le groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia* se développe autant en Petite Camargue qu'aux Rièges quoique ce dernier manque complètement de pin parasol.

Les buissons (*Cistus salviifolius*, *Phillyrea angustifolia*, *Juniperus phoenicea* et d'autres) fortement ombragés par le pin se développent avec moins de vitalité et finissent par périr. Dans l'ombre forte de *Pinus pinea* manquent presque totalement les espèces herbacées. *Crepis bulbosa* seul se développe abondamment sur une couche épaisse de feuilles mortes, dans l'ombre complète. Le *Vulpia longiseta* croît sur des étendues considérables en formant des tapis entiers dans les endroits ombragés pendant une partie de la journée, sur un sol couvert d'une très mince couche de feuilles sèches. L'ombre influence également la phénologie des plantes. La même espèce croissant à l'ombre se développe toujours plus tard qu'à découvert.

Dans la petite forêt de *Pinus pinea* s'accumule d'habitude une couche organique, épaisse de 10 à 30 centimètres (litière non ou peu décomposée). La décomposition avance lentement, mais elle n'arrive jamais à produire de l'humus brut (Rohhumus), parce que l'humus renferme toujours du sable riche en carbonate de calcium, chassé par le vent. D'après nos observations la quantité de CaCO_3 ne descend pas dans cet horizon au-dessous de 5%. Ces conditions expliquent le manque sous le pin parasol (et dans toutes les dunes anciennes) de plantes acidophiles typiques.

9. Remarques sur l'immigration des plantes dans les dunes anciennes

La végétation des dunes anciennes situées à l'Ouest du Petit Rhône, en Petite Camargue et à Silve Godesque, est calcicole. Les pelouses appartiennent à l'ordre des *Thero-Brachypodietalia*. En revanche les espèces végétales et les associations de l'ordre des *Helianthemetalia guttati* fuyantes les calcaires y manquent complètement, bien que les associations de ce dernier ordre se trouvent au voisinage des dunes, sur les gravières pliocènes. Cependant les espèces préférant les sols siliceux y existent.

Cistus salviifolius L. est largement répandu dans toutes les dunes de la Petite Camargue et de la Silve Godesque. Il forme également des touffes parfois même du côté du vent dans les dunes récentes.

Erica scoparia L. n'a été remarquée qu'en un seul endroit dans les dunes au Nord-Est du Grau-du-Roi.

Pteridium aquilinum (L.) Kuhn. se trouve seulement dans les plus anciennes dunes situées au Nord d'Aiguesmortes. Il croît dans la forêt à *Pinus pinea* et *Quercus pubescens* (*Quercetum galloprovinciale*) près du Mas de Trouchaud (Kühnholz-Lordat 1923) et en grande abondance dans les buissons à la Silve Montcalm. Cette fougère croissant le plus souvent sur des sols siliceux, pauvre en carbonate de calcium, ne se trouve qu'exceptionnellement sur des sols calcaires (Litardière 1933).

Silene conica L. est fréquente dans les plus anciennes dunes (Silve Godesque) et fort rare en Petite Camargue (au Sud de Caves de la Pinède).

Les conditions écologiques de développement des forêts à *Pinus pinea* dans les dunes du lido Godesque, où se trouvent déjà 3 espèces de chênes, offrent une grande ressemblance avec les conditions du milieu de ce pin dans les dunes plus jeunes de la Petite Camargue. Il semble qu'en Petite Camargue se trouvent également des dunes suffisamment hautes et stabilisées pour le développement des chênes, puisque *Quercus ilex* et *Q. pubescens* peuvent être rencontrés parfois dans des dunes basses, par exemple à la Silve de Montcalm et aussi dans les dunes récentes. Cependant si on accepterait, que les vieux chênes à racines profondes ne pour-

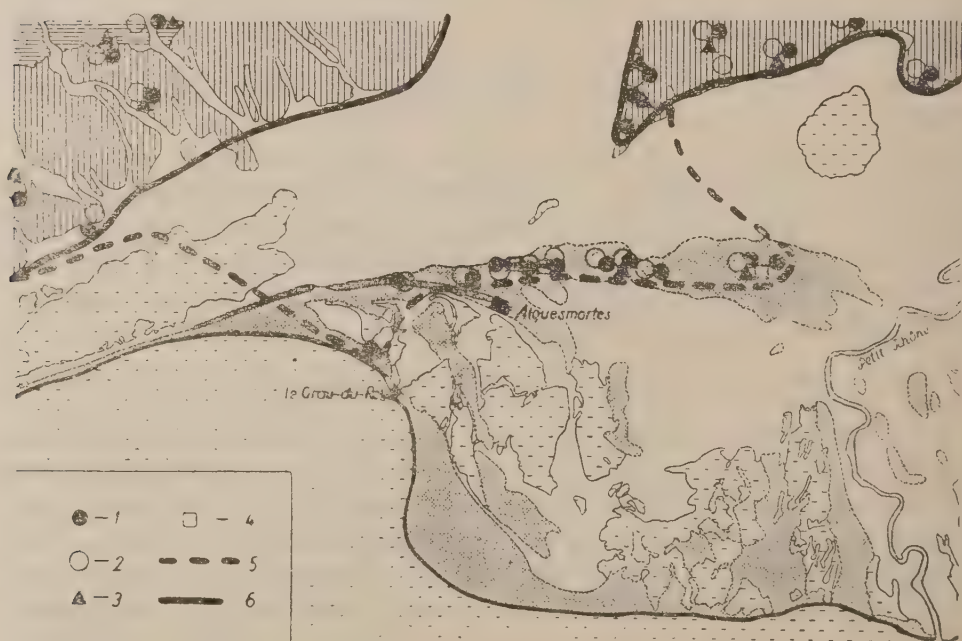


Fig 7. Répartition de certaines plantes à l'Ouest du Petit Rhône: 1 — *Quercus pubescens*; 2 — *Qu. ilex*; 3 — *Qu. coccifera*; 4 — *Pteridium aquilinum*; 5 — limite locale de chênes; 6 — limite locale des myrmécochores et des barochores, qui ont le centre de répartition dans le *Quercetum ilicis galloprovinciale*

raient pas croître en Petite Camargue et aux Rièges à cause du niveau trop élevé de l'eau phréatique, pourquoi n'y trouve-t-on pas de jeunes exemplaires? Ils sont fort abondants dans les forêts à *Pinus pinea* sur les dunes au Nord d'Aiguesmortes. Il est également difficile d'admettre que la distribution des chênes soit empêchée par des causes climatiques, puis-

que *Quercus ilex* arrive jusqu'au bord de la mer. La question se pose, si seules les mauvaises conditions de milieu ont empêché l'installation des chênes et du *Quercetum galloprovinciale* dans les dunes de la Camargue. Pourquoi les individus de cette association situés dans les dunes les plus anciennes au Nord d'Aiguesmortes sont tellement appauvris en comparaison de l'association de chêne vert de la proche formation pliocène? Pour répondre à cette question ne fût-ce qu'en partie, on a analysé la flore des dunes étudiées ici au point de vue de la biologie de la dissémination (tab. 7a et 7b).

Tableau 7b
(Résumé du tableau 7a)

Type de la dissémination	<i>Quercetum ilicis gallopro- vinciale</i>	Terrasse pliocène au Nord d'Aigues- mortes	Dunes les plus anciennes	Dunes anciennes	
			Silve Godesque	Petite Camargue	Bois des Rièges
Nombre d'espèces					
Espèces à dissémination très active					
Anémochores	11	8	6	3	3
Endozoochores	24	24	16	15	15
	35	32	22	18	18
Espèces à dissémination peu active					
Dyszoochores à dias- pores de grandes di- mensions (transport par les oiseaux)	3	3	3	—	—
Espèces à dissémination locale					
Myrmécochores	6	4	—	—	—
Barochores	7	5	—	—	—
Autochores	4	3	1	1	—
	17	12	1	1	—
Nombre d'espèces	55	47	26	19	18

Parmi les 55 espèces abondantes et caractéristiques du *Quercetum galloprovinciale* (Braun-Blanquet 1936) 45 on rencontre sur le pliocène au voisinage des dunes, mais 26 espèces seulement atteignent le lido Godesque, 19 les dunes de la Petite Camargue et 18 seulement se trouvent sur le terrain très isolé des Rièges. Ce sont pour la plupart des endozoochores (ornithochores) et des anémochores, dont les diaspores sont transportées à une grande distance (de plus de 1000 mètres — Moli-

nier et Müller 1938). Des chênes adaptés à la dissémination dys-zoochore se sont également installés sur les dunes de la Silve Godesque. Nous ne trouvons cependant sur aucune des dunes étudiées ni des myrmécochores ni des barochores qui se rencontrent toujours dans les forêts de chêne vert et les garrigues de l'intérieur. Les autochores manquent également à peu près complètement. Fait frappant si l'on considère l'abondance de *Carex Halleriana*, *C. distachya* et *Viola scotophylla* dans les forêts et les garrigues sur pliocène. On pourrait supposer, qu'une bande de terre humide occupée par des marécages forme une barrière infranchissable pour les barochores, les myrmécochores et les autochores, espèces dont les diaspores se disséminent seulement à des petites distances („espèces à dissémination locale, de 0 à 100 mètres" — Molinier et Müller l.c.).

Les stations de chênes (*Quercus ilex*, *Qu. pubescens*, *Qu. coccifera* — fig. 7) dans les dunes de Silve Godesque sont les plus avancés au Sud dans toute la Camargue. Les localités les plus proches de *Quercus ilex* et de *Qu. pubescens*, indiquées par M. Tallon (1930), se trouvent dans la forêt riveraine de Réaumont avec *Ulmus campestris* et *Populus alba* sur un sol sablonneux, élevé, au bord du Petit Rhône, dans la partie Nord du delta.

Nous avons trouvé trois exemplaires buissonneux du Chêne blanc sur les anciennes dunes au Nord-Ouest du Grau-du-Roi pendant une excursion de juin 1959 en compagnie de Madame et Monsieur Braun-Blanquet. C'est la localité située la plus près de la mer, parmi celles qui sont connus en Camargue, et relativement for isolée des autres. Il n'est pas exclu, que cette espèce soit amenée ici par l'homme. On peut également se demander si les chênes des plus anciennes dunes de la Silve Godesque ne sont pas aussi introduits par l'homme et se sont ensuite largement répandus? Cependant cela paraît peu probable. Je n'ai pas observé de chênes cultivés près des mas. Pourquoi d'ailleurs seraient-ils cultivés dans les plus anciennes dunes sans qu'on essaye de les introduire dans l'entourage des maisons en Camargue proprement dite?

Si on accepte — ce qui ne nous paraît juste — que *Quercus ilex* et *Qu. pubescens* se trouvent ici subspontanément, on ne pourra cependant pas croire que *Quercus coccifera* a été introduit et cultivé ici. Il semble donc que les chênes n'ont pas encore réussi à parvenir en Petite Camargue. Les oiseaux transporteurs de semences (les geais surtout) n'ont pas amené les glands la distance étant trop grande. Le noisetier (*Corylus avellana*), disséminé de la même façon, manque également dans ces dunes. Tallon (1931) ne le cite qu'au bord du Rhône (Mas de Toufort) dans la partie Nord de la Camargue.

Etudiant l'immigration des plantes dans le territoire des dunes il convient de se rappeler que la Petite Camargue communique avec l'intérieur

par l'intermédiaire de forêts riveraines des bords du Rhône, qui traverse les terrains salés. On rencontre certaines caractéristiques du *Quercetum ilicis galloprovinciale* dans le *Populetum albae* (Tchou 1948). *Phillyrea angustifolia*, *Ph. media*, *Rubia peregrina* et *Daphne gnidium* croissent actuellement au bord du Petit Rhône aux environs de Sylvéréal.

Beaucoup des caractéristiques de la chênaie à *Quercus ilex* et des garrigues, par exemple: *Asparagus acutifolius*, *Rubia peregrina*, *Clematis flammula* ssp. *flammula*, *Daphne gnidium*, *Pistacia lentiscus*, *Smilax*

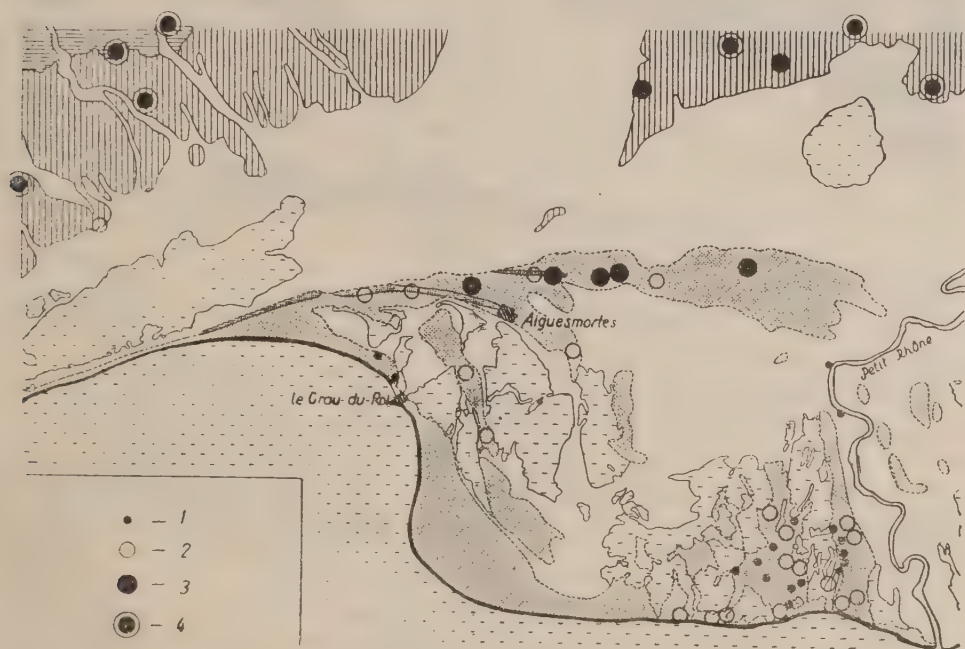


Fig. 8. Nombre des caractéristiques de l'ordre des *Quercetalia ilicis* (*Quercus pubescens* y compris): 1: 1—4; 2: 5—9; 3: 10—12; 4: 13 et plus d'espèces

aspera, *Rhamnus alaternus*, *Lonicera implexa* et *Ruscus aculeatus* s'avancent en Petite Camargue jusqu'au bord de la mer et croissent souvent du côté opposé au vent des dunes récentes. Toutes ces espèces, à l'exception de *Clematis flammula*, qui est un anémochore, sont des espèces ornithochores.

Dans le *Quercetum galloprovinciale pubescentetosum* sur pliocène se trouvent généralement plus de 13 caractéristiques des *Quercetalia ilicis* tandis que les dunes au Nord d'Aiguesmortes n'hébergent en moyenne que 9, et celles de la Petite Camargue en moyenne seulement 5 espèces (fig. 8 at 9).

Les faits que nous venons d'énumérer montrent que l'immigration des végétaux dans les dunes de la Camargue et de la Silve Godesque n'est pas encore terminée. Plus nous nous éloignons du pliocène, plus diminue aussi le nombre des caractéristiques de l'association climax. L'isolement des dunes a fortement retardé l'immigration des plantes. Les alluvions humides et en grande partie couverts d'eau séparant les dunes de l'in-



Fig. 9. Concentration des nombres de caractéristiques de l'ordre *Quercetalia ilicis*. Les places hachurées en gradation marquent où ils se montrent: 1: 1—9; 2: 10—12; 3: 13 et plus d'espèces

térieur jouent le rôle de barrière biologique. Certaines espèces ne peuvent probablement pas franchir cette barrière et atteindre les dunes, bien que beaucoup auraient sans doute trouvé des conditions favorables à leur installation.

Des faits analogues sont déjà indiqués. „Dass Wasserflächen, sogar von einigen Kilometern Ausdehnung, wichtige Barrieren bilden können, hat Palmgren für Åland gezeigt. [...] man kann aus der Untersuchung Palmgrens und anderer den Schluss ziehen, dass die Besiedlung eines Bodens sich über Jahrtausende ausdehnt” (Lundegårdh 1957).

Dans la première période de l'envahissement des dunes par la végétation la succession progresse très vite. Les différents stades se succèdent rapidement. Plus le développement de la végétation est avancé, plus le

procès de l'immigration de nouvelles espèces devient lent et difficile et la composition floristique change si lentement qu'elle est presque imperceptible.

RÉSUMÉ ET CONCLUSIONS

Le développement de la végétation dans les dunes anciennes (de sable riche en calcaire), de la Camargue mène à l'installation de l'association forestrière (climax), le *Quercetum ilicis galloprovinciale*. Ce procès, dont le schéma a été présenté par Braun-Blanquet (1936), comprend ici les stades suivants: *Agropyretum* → *Ammophiletum* → *Crucianellum* → (groupement à *Artemisia campestris* var. *glutinosa*) → groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia* → stade à *Quercus ilex* et *Qu. pubescens* (*Quercetum ilicis galloprovinciale* appauvri) → *Quercetum ilicis galloprovinciale*.

Tableau 8

La liste des groupements végétaux se développant dans les dunes de Petite Camargue et de la Silve Godesque

L'ordre	Dunes anciennes (Petite Camargue)	Dunes les plus anciennes (Silve Godesque)
<i>Ammophiletalia</i>	<i>Ammophiletum</i> (fragment.)	
<i>Thero-Brachypodietalia</i>	<i>Staticetum echinoidis</i>	<i>Staticetum echinoidis</i> (appauvri)
<i>Quercetalia ilicis</i>	Groupement à <i>Juniperus phoenicea</i> var. <i>lycia</i> et <i>Phillyrea angustifolia</i> de l'alliance <i>Quercion ilicis</i>	<i>Quercetum ilicis galloprovinciale</i> (appauvri)
<i>Populetales albae</i>	manque complet	<i>Populetales albae</i>

Nous donnons la liste (tab. 8) des groupements végétaux se développant dans les dunes en Petite Camargue et de la Silve Godesque.

Quercetum galloprovinciale appauvri se développe déjà dans les dunes de la Silve Godesque. Les dunes plus jeunes de la Petite Camargue sont occupées sur une étendue considérable par le groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia*. Les associations dunales (de l'ordre des *Ammophiletalia*) et les pelouses calcaires (de l'ordre des *Thero-Brachypodietalia*) se développent un peu mieux en Camargue qu'à Silve Godesque. C'est le contraire pour la végétation riveraine. Dans les dépressions parmi les dunes les plus anciennes existent des fragments du *Populetales albae*. En Petite Camargue les dépressions sont occupées

par la végétation halophile, tandis que les plantes propres aux forêts riveraines y manquent complètement (fig 10 et 11).

L'immigration des plantes dans les dunes anciennes de la Camargue n'est pas encore achevée, malgré leur âge avancé. L'envahissement des

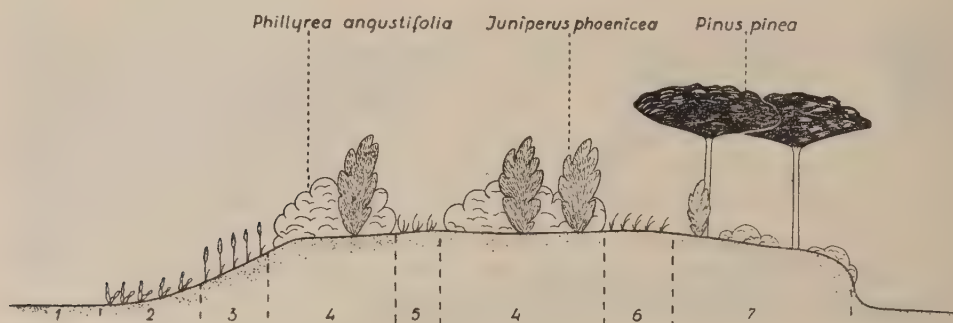


Fig. 10. Coupe schématique à travers les dunes anciennes en Petite Camargue (près de la Maison le Ferradou): 1 — sol nu; 2 — les groupements de l'ordre des *Salicornietalia*; 3 — les groupements de l'ordre des *Juncetalia maritimi*; 4 — groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia*; 5 — *Staticetum echinoidis* typique; 6 — *Staticetum echinoidis* variante à *Vulpia longiseta*; 7 — groupement à *Juniperus phoenicea* var. *lycia* et *Phillyrea angustifolia* variante à *Pinus pinea*

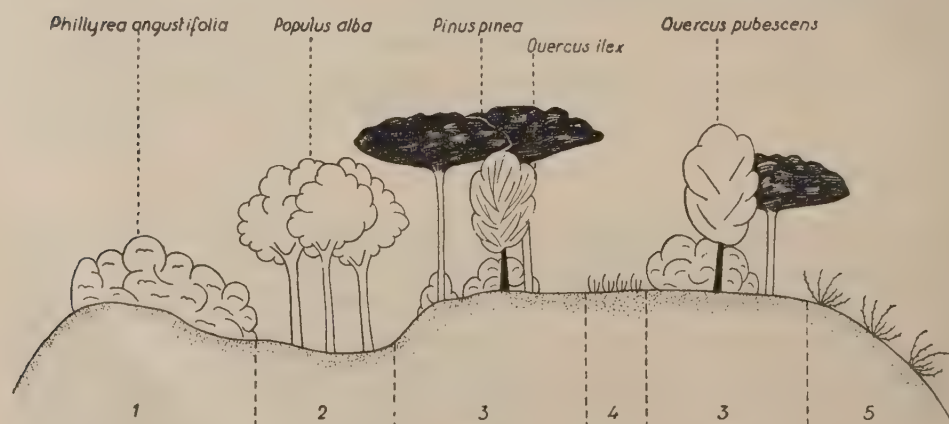


Fig. 11. Coupe schématique à travers les dunes les plus anciennes (Silve = lido Godesque) situées au Nord d'Aiguesmortes (près du Mas de l'Abbé): 1 — buissons à *Phillyrea angustifolia*; 2 — *Populetum albae*; 3 — *Quercetum ilicis galloprovinciale* (appauvri) avec *Pinus pinea*; 4 — *Staticetum echinoidis* variante à *Vulpia longiseta*; 5 — groupement à *Artemisia campestris* var. *glutinosa*

dunes étudiées par l'association de chêne vert se poursuit très lentement. On suppose qu'en Petite Camargue et à Silve Godesque la lenteur de la succession n'est pas causée seulement par des conditions du milieu, mais aussi par les difficultés qui s'opposent à l'arrivée des diaspores de nombreuses plantes. Beaucoup d'espèces forestières, surtout barochores, myrmécochores et autochores ont rencontré une barrière presque infranchissable dans une bande humide d'alluvions et l'eau séparant les dunes de pliocène portant le *Quercetum galloprovinciale*. Les chênes (*Quercus ilex*, *Qu. pubescens* et *Qu. coccifera*), adaptés à la dissémination dyszoochore ont franchi cette barrière et sont arrivés aux dunes situées au Nord d'Aiguesmortes (le lido Godesque), mais manquent complètement dans les dunes plus jeunes de la Petite Camargue et aux Rièges, très isolés.

L'influence de *Pinus pinea* sur le développement de la végétation et du sol des dunes n'est pas si considérable que l'on pourrait le supposer. Dans les forêts de pin s'accumule d'habitude une couche de litière non ou peu décomposée, épaisse de 10 à 30 centimètres, mais cela n'aboutit pas à la formation d'humus brut, parce que la litière contient toujours du sable riche en CaCO_3 chassé par le vent. C'est la raison pourquoi ces dunes ont une végétation calcicole et même sous le pin parasol n'apparaît aucune plante nettement acidophile. Cependant *Cistus salviifolius*, *Pteridium aquilinum*, *Erica scoparia* et *Silene conica*, espèces préférant le sol sili-
ceux y existent.

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STRESZCZENIE

Stare wydmy śródlądowe, pochodzenia morskiego, położone w delcie Rodanu (Camargue), na prawym brzegu Małego Rodanu (Petit Rhône), dadzą się podzielić na dwa typy, w zależności od ich wieku. Najstarszy kordon litoralny, zwany lido lub Silve Godesque (jego wiek ocenia się na blisko 5000 lat), położony jest daleko w głębi łądu. Młodsze i bliższe morza są wydmy Małej Camargue (Petite Camargue). Gruboziarnisty piasek wydmy zawiera 20—30% węgla wapnia. Wydmy są wyraźnie oddzielone pasem aluwii (częściowo zalanych wodą, a częściowo zasolonych) od starszych utworów geologicznych (fig. 1).

Rozwój roślinności na wydmach Camargue prowadzi do zainstalowania się na nich klimaksowego zespołu leśnego z dębem wieczniezielonym (*Quercetum ilicis galloprovinciale*). Proces ten, za Braun-Blanquet'em (1936), można przedstawić następującym schematem: *Agropyretum* → *Ammophiletum* → *Crucianelletum* → (Zbiorowisko *Artemisia campestris* var. *glutinosa*) → Zbiorowisko *Juniperus*

phoenicea var. *lycia* i *Phillyrea angustifolia* → Stadium *Quercus pubescens* i *Qu. ilex* (zubożałe *Quercetum galloprovinciale*) → *Quercetum ilicis galloprovinciale*.

Na wydmach najstarszych (Silve Godesque) rozwijają się już zubożałe płaty zespołu *Quercetum ilicis galloprovinciale* (tabela 4 oraz fig. 10). Młodsze od nich wydmy Petite Camargue na znacznych przestrzeniach porasta zbiorowisko *Juniperus phoenicea* var. *lycia* i *Phillyrea angustifolia* (tab. 3a i 3b; fig. 9), wyraźnie słabiej zaawansowane w sukcesji. Zespoły wydymowe (z rzędu *Ammophiletalia* str. 533 oraz tab. 1) i zespoły śródziemnomorskich muraw wapieniolubnych (z rzędu *Thero-Brachypodietalia*, tab. 2) znajdują korzystniejsze nieco warunki rozwoju na wydmach młodszych i wykształcają się lepiej niż na wydmach najstarszych. Odwrotnie ma się sprawa z roślinnością łągową. W zagłębieniach śródwydmowych wydmy najstarszych rozwija się już fragmentarycznie zespół *Populetum albae* (str. 594), gdy w obrębie wydmy Małej Camargue i Rièges zagłębienia zajmuje roślinność halofilna, a gatunków charakterystycznych dla lasów łągowych brak w ogóle, ze względu na zasolenie wód gruntowych.

Migracja roślin na stare wydmy Camargue nie została jeszcze zakończona, mimo ich znacznego wieku. Na powolne tempo sukcesji wpłynęły zarówno nie sprzyjające warunki edaficzne, jak i trudność dostania się na wydmy diaspor wielu gatunków roślin. Wilgotny pas aluwium oddzielający wydmy od żwirów pliocenskich, na których rozwija się zespół dębu wieczniezielonego (*Quercetum galloprovinciale pubescentosum*), jest najprawdopodobniej dla wielu charakterystycznych dla tego zespołu gatunków roślin rozprzestrzeniających się jako barochory, myrmekochory i autochory niemożliwą niemal do sforsowania przeszkodą biologiczną (tab. 7a, 7b). Dyszoochorycznie rozprzestrzeniające się dęby (*Quercus ilex*, *Qu. pubescens* i *Qu. coccifera*) zdołały mimo tej bariery dotrzeć na wydmy najstarsze (fig. 7), brak ich natomiast zupełnie na wydmach młodszych, w Petite Camargue i w Rièges.

Wpływ pinii (*Pinus pinea*) (o której nie wiadomo, czy występowała z natury na wydmach w delcie Rodanu, czy też została tu niegdyś wprowadzona przez człowieka) na rozwój roślinności i gleb wydmych nie jest wielki. W laskach piniowych gromadzi się wprawdzie zazwyczaj 10–30 cm gruba warstwa nie rozłożonych igieł, nigdy nie dochodzi jednak do wytworzenia kwaśnej butwiny, ponieważ stale zasypywana jest ona piaskiem zasobnym w CaCO_3 . Ilość węgla wapnia nie spada poniżej 5%, a pH (mierzone w H_2O) poniżej 6,6 (tab. 6), stąd roślinność badanych wydmy, tak najstarszych jak i młodszych, ma charakter roślinności wapieniolubnej; nawet pod piną nie zjawiają się typowo acidofilne gatunki roślin. Na wydmach tych zdarzają się natomiast gatunki, które wprawdzie nie unikają podłoża zasobnego w wapień, ale chętniej rosną na podłożu bezwapiennym (*Cistus salviifolius*, *Erica scoparia*, *Pteridium aquilinum* i *Silene conica*).

Materiały do niniejszej publikacji zebrane zostały w czasie 3½-miesięcznego pobytu autora w Międzynarodowej Stacji Geobotanicznej (SIGMA) w Montpellier, której dyrektorowi, prof. drowi J. Braun-Blanquet autor wyraża serdeczne podziękowanie za wskazanie tematu, kierownictwo naukowe i okazowaną życzliwość; dziękuje on również Wydziałowi II Nauk Biologicznych Polskiej Akademii Nauk za przyznanie stypendium, które umożliwiło wyjazd do Francji.

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de Géobotanique Méditerranéenne et Alpine,
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w Krakowie.*

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The heredity of specific traits in the hybrid *Mirabilis jalapa* × *Mirabilis longiflora*

by

ANNA KRUSZEWSKA

The genus *Mirabilis*, in particular the species *M. jalapa* and *M. longiflora* have been the subject of many investigations. The great morphological differences between these two species and especially the difference in the length of the flower tube have for long attracted the attention of genetists.

The first crosses between *M. jalapa* and *M. longiflora* were obtained as early as the 18th and the 19th century by Kölreuter (1764), Gärtner (1849), Lecoq (1862), and Naudin (1863).

Even those early investigators noticed that hybrids between these two species could be obtained only when *M. jalapa* was the maternal plant.

The first more detailed analysis of the hybrids *M. jalapa* × *M. longiflora* was carried out by Correns (1902) who described the manner in which the flower colour, the tube length, and the pollen size were inherited in the F_1 progeny of these species.

A still more complete analysis of the inheritance of some specific traits accounting also for the F_2 generation of this hybrid was carried out by Prakken (1944). He studied in particular the heredity of the length of the flower tube, the flower colour, and the pubescence.

The cytological study of the genus *Mirabilis* is rather difficult. The originally established chromosome number for some species in this genus has been subsequently corrected more than once.

The course of meiosis in the F_1 hybrids *M. jalapa* × *M. longiflora* and in the parental species was studied and compared by Showalter (1935) and Prakken (1944). According to Showalter the reduction divisions in both pure species and in the F_1 hybrid are strongly disturbed. However, Prakken is of the opinion that in the pure species meiosis proceeds regularly and undisturbed, and that disturbances in the reduction divisions of the F_1 hybrids are few.

Earlier descriptions of the heredity of specific traits in the hybrids *M. jalapa* × *M. longiflora* were restricted to only a few of the numerous

traits distinguishing these interesting species, whereas some results of the cytological investigations were contradictory. For this reason I have thought useful to repeat in greater detail the genetic analysis of the differences between the two species and to extend the investigation over a greater number of morphological traits and some physiological traits.

Meiosis in *M. jalapa* and in the F_1 hybrid *M. jalapa* $\times$ *M. longiflora* were also studied.

MATERIAL AND METHODS

The plants used for the crosses were obtained from seeds of plants grown for many generations in the Botanical Garden in Warsaw.

M. jalapa was the maternal plant, for, as has been mentioned, all reciprocal crosses were unsuccessful.

The setting of seeds when *M. jalapa* was pollinated with pollen of *M. longiflora* was very poor.

Although very many flowers were pollinated one seed only was found and from this seed one F_1 plant was grown. This cross was obtained by A. Doroszevska from whom I received the F_1 plant and some seeds for F_2 . The whole F_2 generation was the progeny of this one F_1 plant. The seeds for F_2 were obtained by spacial isolation of the F_1 plant, since the setting of seeds under the usual isolating bags was much lower.

Numerous flowers of *M. jalapa* were also pollinated with pollen of the F_1 hybrid, but only two seeds were obtained from which one plant germinated.

From the reciprocal backcross not one seed was obtained.

Similarly all attempts at backcrosses with *M. longiflora* were unsuccessful.

Except for the F_1 plant all the plants of *M. jalapa*, *M. longiflora*, and the F_2 generation were grown close to each other on the same plot under similar conditions. The measurements were carried out during two successive seasons.

For the examinations of meiosis in pollen mother cells the buds were fixed in Navaschin's fixative according to Randolph's modification, embedded in paraffin, cut into sections 15 μ thick, and stained with crystal violet.

Pollen fertility was calculated after staining with aceto-carmin. About 400 pollen grains from each plant were examined and the percentage of grains with well stained cytoplasm and nuclei was counted.

CYTOLOGICAL ANALYSIS

The course of meiosis was examined in PMCs of *M. jalapa* and the F_1 hybrid.

Conformably with what was reported by Showalter and Praken the chromosome number is $n=29$ (Fig. 1).

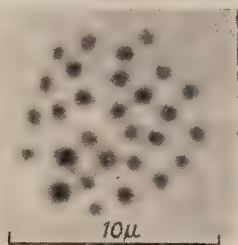


Fig. 1. Metaphase of the first meiotic division with 29 bivalents in *M. jalapa* — polar view

The course of meiosis in *M. jalapa* was not entirely regular. In the 30 examined PMCs in the stages of metaphase I, anaphase I, or telophase I such disturbances as single univalents and lagging chromosomes were found in three instances (Fig. 2).

In the F_1 hybrid the disturbances of meiosis are more frequent. In the 32 examined PMCs at the stages of metaphase I, anaphase I, or telophase I irregularities of meiosis occurred in as many as 17 cells (Fig. 3).

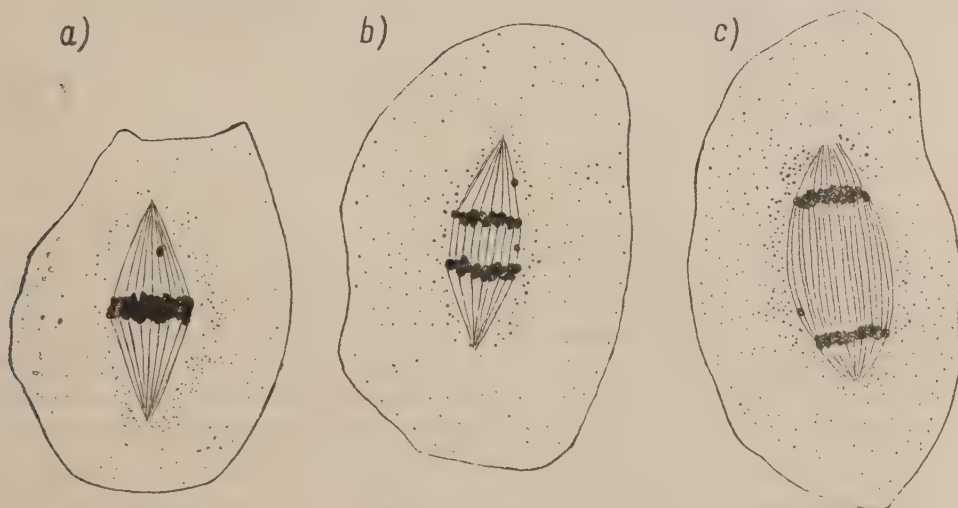


Fig. 2. Disturbances of meiotic divisions in *M. jalapa*. a — Metaphase I (side view) with a single chromosome, probably a univalent, lying between the equator and the pole of the spindle; b — Anaphase I (side view) with two chromosomes lying separately; c — Late anaphase I (side view) with a chromosome lagging in the spindle ($\times 3000$)

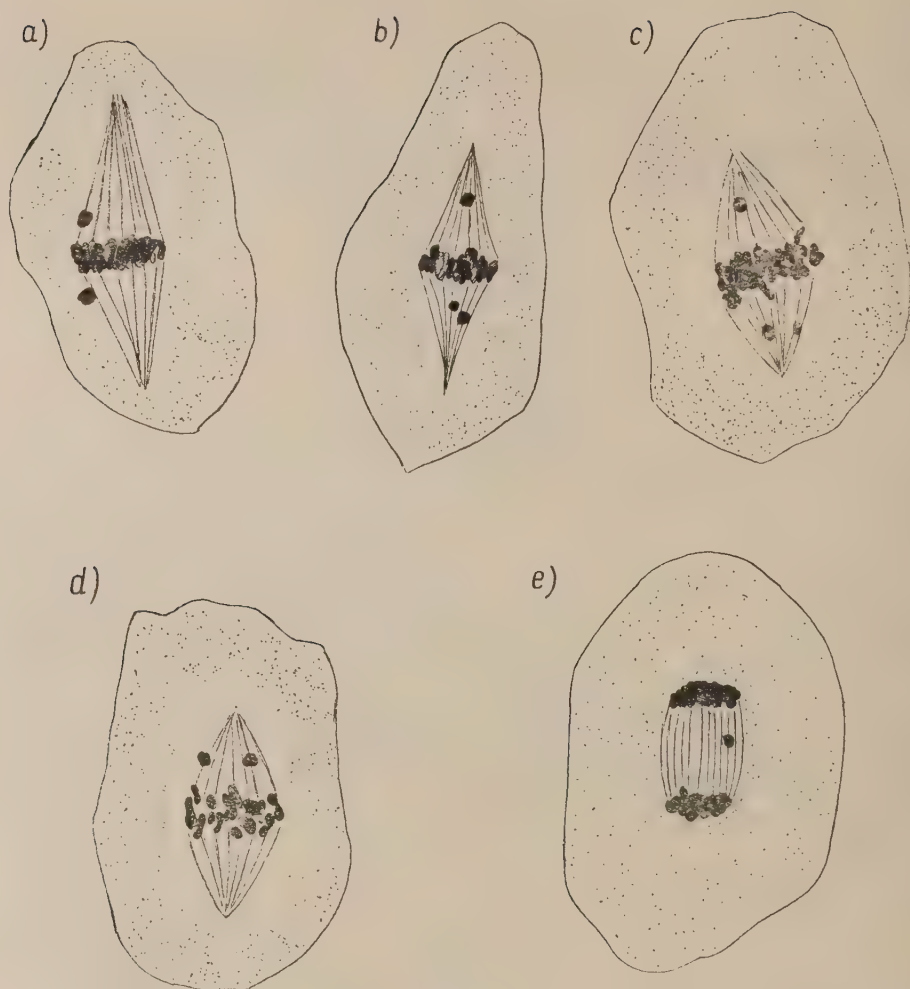


Fig. 3. Disturbances of meiotic divisions in the F_1 hybrid *M. jalapa* $\times$ *M. longiflora*. a — Metaphase I (side view) with two large chromosomes on either side of the equator; b — Metaphase I (side view) with three univalents, two large and one small; c — Early anaphase I (side view) with two groups of segregating chromosomes still on the equatorial plate and three chromosomes lying apart already close to the poles of the spindle; d — early anaphase I (side view) with two large chromosomes lying far from the others; e — Telophase I (side view) with a chromosome lagging on the spindle ($\times 3000$)

In metaphase I the disturbances consisted in the presence of univalents, usually two large ones, lying at some distance from the other chromosomes grouped on the equatorial plate of the spindle. In anaphase I and in telophase I there were chromosomes, probably univalents, lagging on the equatorial plate of the spindle.

GENETICAL ANALYSIS

The following traits were measured and defined for the genetic analysis:

1. Length of flower tube.
2. Length of style.
3. Length of the lobe of the corolla.
4. Width of the lobe of the corolla.
5. Length of the lobe of the calyx.
6. Width of the lobe of the calyx.
7. Growth habit.
8. Morphology of leaves.
9. Pubescence on stems, leaves, and flowers.
10. Colour of stems.
11. Colour of flowers.
12. Date of flowering.
13. Time of anthesis.
14. Pollen fertility.

1. Length of flower tubes

The fused petals of the corolla of the flowers in *Mirabilis* form a narrow tube expanding at the end in five radially arranged lobes.

The length of the flower tube is the main specific trait distinguishing *Mirabilis jalapa* and *Mirabilis longiflora*. The tubes were measured from the end of the ovary to the point at which they form lobes (Fig. 4).

Table I lists the lengths in millimetres of flower tubes in the parental species, F₁ and F₂.

The row marked *Mj* lists the flower tube lengths in *M. jalapa* with white and red flowers only. The flower tube lengths in these two varieties are approximately the same averaging at about 31.1 mm. The parental plant in this investigation was *M. jalapa* with red flowers. The variety with variegated flowers which has flower tubes shorter than 30.0 mm., average about 25.0 mm., was not considered.

The average length of flower tubes in F₁ was 53.9 mm. and was much less than the arithmetic mean of tube lengths for the two parental species, which was 79.0 mm. This means that F₁ is distinctly shifted towards *M. jalapa*.

In the F₂ generation flower tubes were measured in 252 plants, ten flowers in each. In this generation the variation practically did not extend beyond the variation range in F₁ on the side of *M. longiflora*. Not one

plant was found in which the mean length of flower tubes from ten flowers was more than 65.0 mm., whereas the average length of tubes in individual plants of *M. longiflora* ranged 115.0 to 130.0 mm., and the

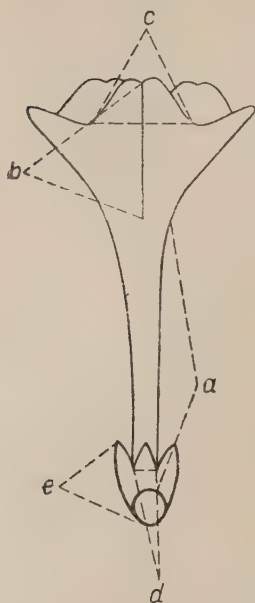


Fig. 4. Schematic diagram of *Mirabilis* flowers with the traits measured in the investigation marked in: *a* — Length of flower tube; *b* — Length of the lobe of the corolla; *c* — Width of the lobe of the corolla; *d* — Width of the lobe of the calyx; *e* — Length of the lobe of the calyx

length of tubes in individual flowers was 105.00 to 140.00 mm. On the other hand in the F_2 generation there were 23 plants with flower tubes of the same average length as in *M. jalapa*.

As is to be seen from these data the variation in the F_2 generation has a continuous character and a normal distribution, but is distinctly shifted towards the smaller values. Including the variation in *M. jalapa* the variation in F_2 does not attain the values for the other parental species. The average of the tube lengths for the whole of the F_2 generation is even lower than in F_1 which also is very characteristic.

Since the growth of plants essentially consists in the multiplication of living matter it is more convenient to express the relations here considered on a graph with a logarithmic scale.

Fig. 5 shows on the logarithmic scale the mean lengths of the flower tubes plotted against the number of plants in intervals of 0.02 log.

The graph with the logarithmic scale has been plotted so as to check whether the abnormal shift of the variation in F_1 and F_2 towards *M. jalapa* was not caused by some error in the choice of the arithmetic scale.

However, as is to be seen, even on the logarithmic graph the variation of the flower-tube length is shifted in F_2 towards *M. jalapa*, though in this case the shift is not as marked as in the table.

Table 1
Length of flower tubes in mm

Class values	25	30	35	40	45	50	55	60	65	70	75	80	85	90	95	100	105	110	115	120	125	130	N	$\bar{x}$	S	s_x^2
M.j.		2	10																				12	31,1	2,0	0,56
M.l.																							5	122,9	4,8	2,1
F ₁							1												1				1	53,9		
F ₂		5	18	55	72	52	33	14	3														252	43,6	7,2	0,45

Table 2
Length of the lobe of the calyx in mm.

Class values	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	N	$\bar{x}$	S	s_x^2
M.j.																9	16,1	0,57	0,19
M.l.																5	14,9	0,34	0,15
F ₁																1			
F ₂		1	21	38	47	33	42	17	12	13	9	6	5	7	1	252	12,0	2,7	0,17

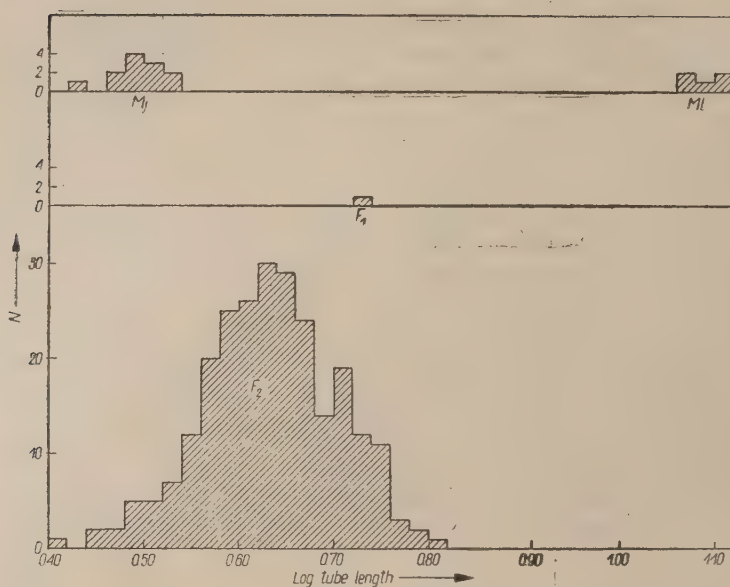


Fig. 5. Frequency histogram of the logarithm of the average flower tube lengths in *M. jalapa*, *M. longiflora*, F_1 , and F_2

2. Length of style

The great difference in the length of flower tubes between the two parental species is associated with a similar difference in the length of the styles.

The average lengths of styles for five flowers in each of nine *M. jalapa* plants ranged 44.0 to 55.2 mm., the average for the nine plants being 47.7 mm.

The corresponding values for five *M. longiflora* plants were respectively 127.8 to 137.2 mm. and 132.8 mm.

The average length of styles for 100 flowers in the F_1 plant was 67.2 mm.

In the F_2 generation the length of styles was measured in 198 plants, in five flowers on each. The average length of styles in the particular plants ranged 40.0 to 90.0 mm.

The variation range of this trait in the parental species and the hybrids is shown on the logarithmic graph (Fig. 7). This graph resembles the one in fig. 5.

The variation in the F_2 generation is continuous and has a normal distribution, but similarly as in the case of the flower tube lengths it is shifted towards lower values including on one side the variation of

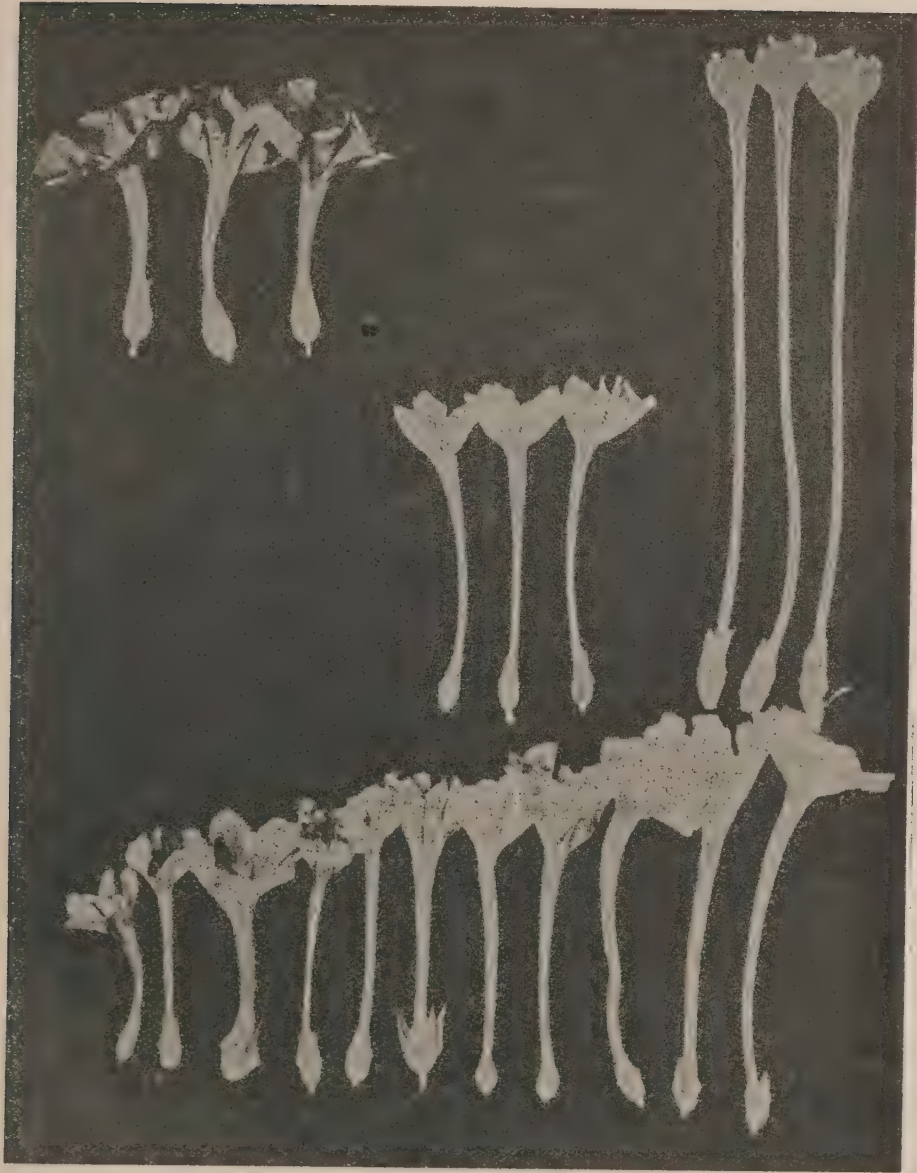


Fig. 6. Photograph of flowers of *M. jalapa* (top left), *M. longiflora* (top right), F_1 (middle), and F_2 (bottom). The first flower on the left in the bottom row is from the F_2 plant with the shortest flower tube and the first flower on the right is from the F_2 plant with the longest flower tube

M. jalapa and not extending on the other to the variation of *M. longiflora*. However, the difference between the variations in *M. longiflora*

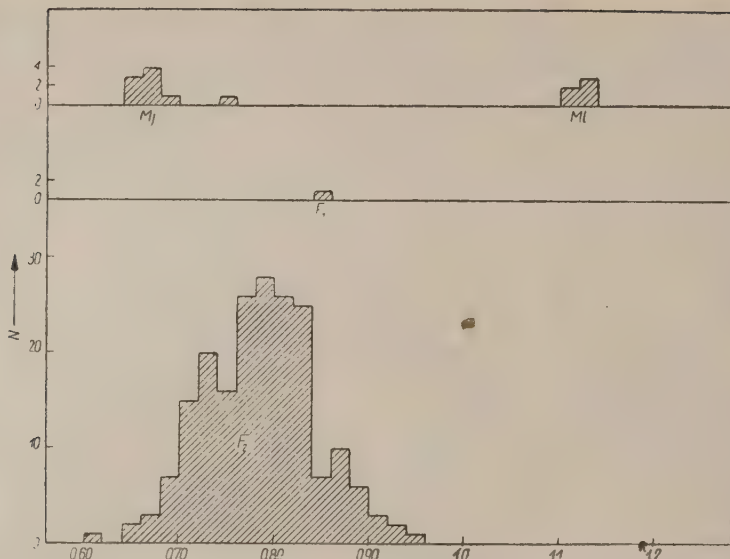


Fig. 7. Frequency histogram of the logarithm of the average style lengths in *M. jalapa*, *M. longiflora*, F_1 , and F_2

and in the F_2 hybrids is somewhat smaller in the case of the length of styles than of the flower tubes.

3. Length of the lobe of the corolla

The lobes were measured from the point at which the flower tubes began to expand suddenly (fig. 4 b).

So far as the length of the lobe is concerned *M. jalapa* and *M. longiflora* differ only slightly. On the average the lobes in *M. longiflora* are somewhat shorter.

The lobes of ten flowers in each of nine *M. jalapa* plants were measured and the average lengths for the particular plants ranged 19.50 to 24.00 mm. The average length of the lobe for all the nine plants was 22.06 mm.

The average lengths of lobes in five *M. longiflora* plants ranged 17.30 to 20.90 mm. The average length of lobes of all the five plants was 19.30 mm.

The average length of lobes of 100 flowers in the F_1 plant was 23.50 mm.

In the F_2 generation the lobes were measured in 252 plants, in ten

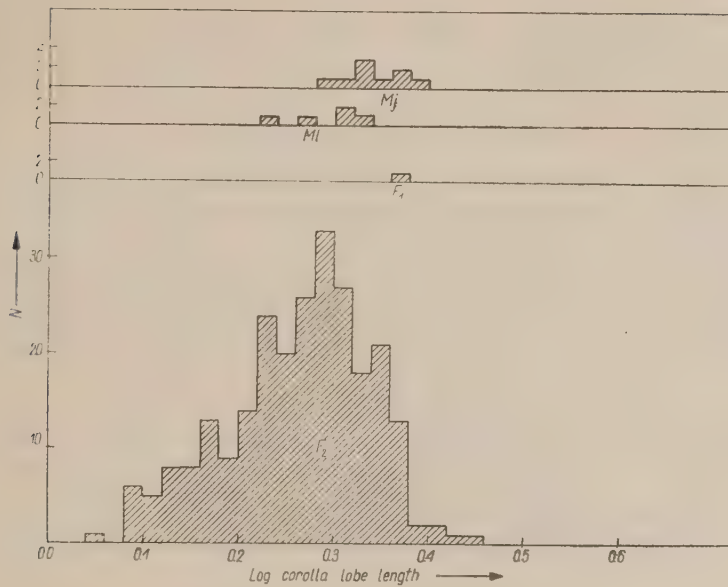


Fig. 8. Frequency histogram of the logarithm of the average lengths of the corolla lobes in *M. jalapa*, *M. longiflora*, F_1 , and F_2

flowers on each. The average length of lobes for the particular plants ranged 11.10 to 28.00 mm. This means that the variation in F_2 extends somewhat beyond the variation in the two parental species.

The variation ranges of the lobe length in the parental species and the hybrids are illustrated by the logarithmic graph in fig. 8.

4. Width of the lobe of the corolla

The difference between *M. jalapa* and *M. longiflora* in the width of the lobes is well marked.

The average widths of the lobes of ten flowers in each of nine *M. jalapa* plants ranged 21.40 to 26.60 mm.

The corresponding average values for the lobes of the flowers in five *M. longiflora* plants were 15.00 and 17.50 mm.

The average width of lobes of 100 flowers in the F_1 plant was 18.00 mm.

In the F_2 generation the width of lobes was measured in 252 plants, in ten flowers on each. The average width in the particular plants ranged 9.20 to 23.00 mm.

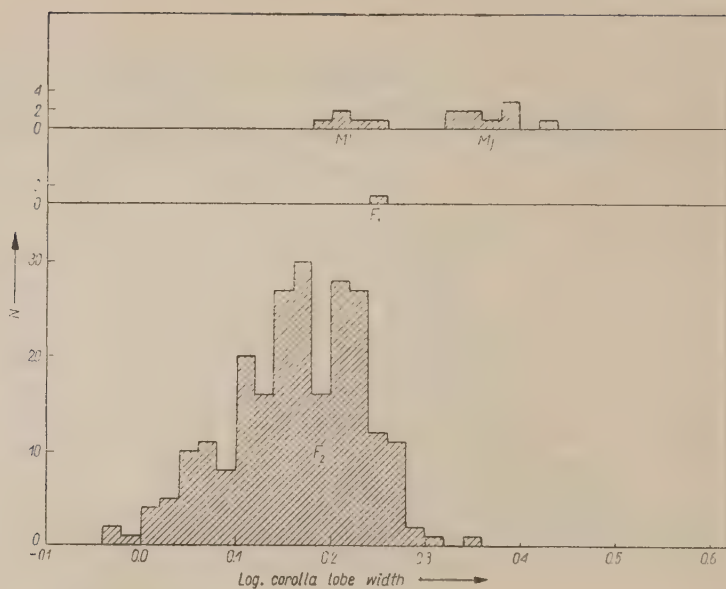


Fig. 9. Frequency histogram of the logarithm of the average widths of the corolla lobes in *M. jalapa*, *M. longiflora*, F_1 , and F_2

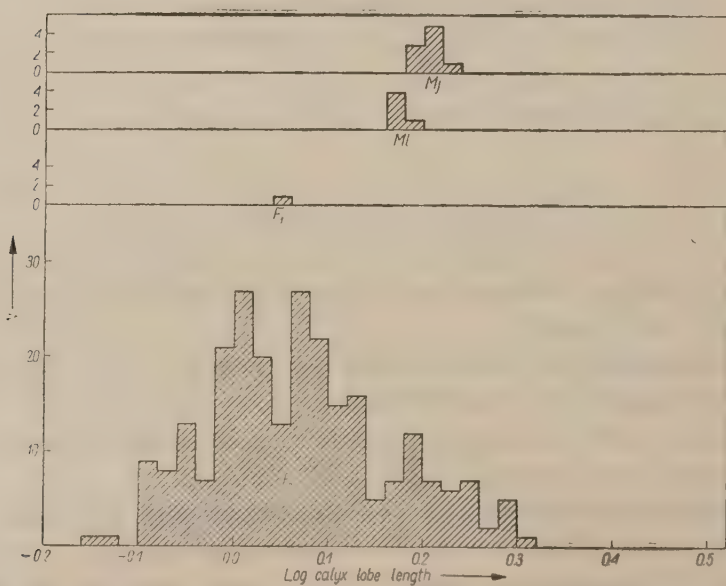


Fig. 10. Frequency histogram of the logarithm of the average lengths of the calyx lobes in *M. jalapa*, *M. longiflora*, F_1 , and F_2

These values indicate a certain degree of transgression beyond the values of *M. longiflora*. The transgression is clearly visible on the logarithmic graph in fig. 9.

5. Length of the lobe of the calyx

(Morphologically this is not the true calyx but calyx-like bractlets)

The difference between the lengths of the lobes of the calyx in the two parental species is small.

Table 2 shows the variation of this trait in *M. jalapa*, *M. longiflora*, F_1 and F_2 .

The well marked transgression in the F_2 generation proves that the length of the lobes of the calyx is controlled by many polymerous genes.

The transgression in F_2 with regard to both parental species especially visible on the side of *M. longiflora*, is also reflected by the graph showing the range of variation of this trait on the logarithmic scale (Fig. 10).

6. Width of the lobe of the calyx

Although the difference in the lengths of lobes between *M. jalapa* and *M. longiflora* is small the two species distinctly differ by the width of the lobes. In *M. jalapa* they are much wider than in *M. longiflora*.

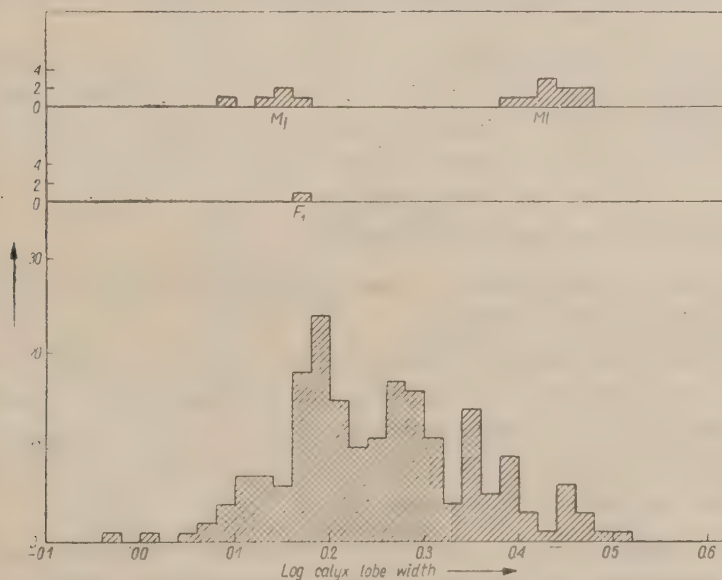


Fig. 11. Frequency histogram of the logarithm of the average widths of the calyx lobes in *M. jalapa*, *M. longiflora*, F_1 , and F_2

Err.: The signes Mj and Ml on the fig. 11 should be interchaned.

In order to obtain greater values the widths of all the lobes in each flower were added up and the average total width of the lobes in five flowers, i.e. the average circumference of the calyx of five flowers, of every plant was compared.

The average circumference of the calyx in nine *M. jalapa* plants ranged 24.2 to 31.2 mm. and in five *M. longiflora* plants 13.0 to 15.0 mm.

The analogical average for 20 flowers of the F_1 plant was 14.8 mm. The average circumferences of the calyx of 198 F_2 plants ranged 9.3 to 32.2 mm.

The graph in fig. 11 shows these values on the logarithmic scale.

7. Growth habit

M. jalapa and *M. longiflora* strikingly differ in their growth habits. These differences consist in the direction of growth of the main stem, the direction of the main branchings, the number and the thickness of the main branches, the number of nodes, and the length of internodes.

The most important of these traits is the direction in which stems grow and that trait was studied in greater detail. Further on in this paper when referring to the growth habit I am thinking only of the direction in which stems grow.

The main stems of *M. jalapa* grow straight up and at 2 to 10 cm. above the ground they branch giving rise to the main branches, which also manifest a negative geotropism. The main branches give rise to numerous ramifications forming a compact crown.

It is characteristic for this species that above 2 to 10 cm. the main stem becomes almost undistinguishable.

The height of the *M. jalapa* plants almost equals their diameter and is on the average about 70 cm.

M. longiflora has the main stem and the side stems of the first order creeping on the ground. The ramifications of higher orders rise slightly and the smallest twigs grow vertically.

In contrast with *M. jalapa* the main stem in *M. longiflora* is very distinct. Its average length is about 120 cm.

Unlike *M. jalapa* the plants of *M. longiflora* are very outspread. Their diameter is as much as 150 cm., and their height about 40 cm.

The F_1 plant is very vigorous. Its growth habit is semi-prostrate resembling more *M. longiflora* than *M. jalapa*. Most main branches lie on the ground but some of them rise slightly.

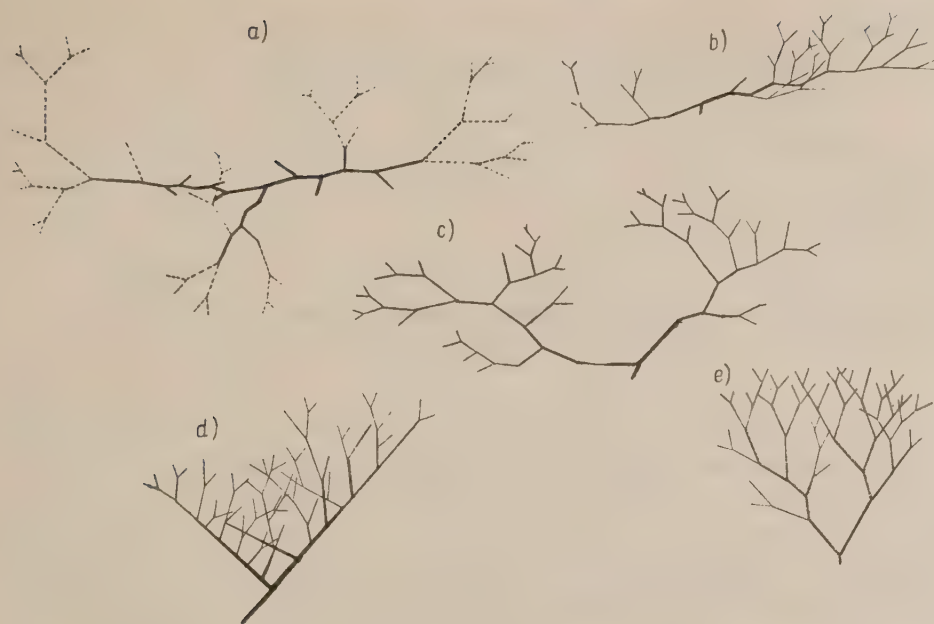


Fig. 12. Schematic diagram of the growth habits in the F_2 generation of the hybrid *M. jalapa* × *M. longiflora*. a — Growth habit of type I F_2 plants and of *M. longiflora*; b — Growth habit of type type II F_2 plants and of the F_1 plant; c — Growth habit of type III F_2 plants; d — Growth habit of type IV F_2 plants; e — Growth habit of type V F_2 plants and of *M. jalapa*

In the F_2 generation of 274 individuals the following types of growth habits were distinguished (Fig. 12):

- I. Growth habit as in *M. longiflora*.
- II. Growth habit semi-prostrate, resembling *M. longiflora*, more or less as in the F_1 plant.
- III. Growth habit intermediate.
- IV. Growth habit approaching *M. jalapa*, the main stem not vertically upright as in *M. jalapa* but usually a little slanting.
- V. Growth habit as in *M. jalapa*.

The numbers of plants in the particular classes are listed in table 3. The mutual ratio of the numbers in the particular classes is approximately 3 : 6 : 4 : 2 : 1.

The deviations of this ratio from the theoretical values are very small. The values of X^2 is 1.00, which for four degrees of freedom makes the value p between 90 and 95 per cent.

The segregation as shown by the ratio stated above leads to the conclusion that the trait of the growth habit is determined by two indepen-

Table 3

The inheritance of the growth habit

Class values	I	II	III	IV	V	N
M. j.					+	
M. l.	+					
F ₁		1				
F ₂ Observed	49	97	74	36	18	274
Expected	51.4	102.8	68.5	34.2	17.1	274
Ratio	3	6	4	2	1	

dent pairs of alleles. The genes *A* and *B* determine the prostrate growth habit, whereas their alleles *a* and *b* determine the upright growth habit. The gene *B* of the prostrate growth habit is dominant over the gene *b*, but neither gene of the other pair is dominant over its allele. Consequently *M. longiflora* is homozygous with regard to both the prostrate growth habit genes and its genotype is *AABB*, whereas *M. jalapa* is homozygous with regard to both the upright growth habit genes and has the genotype *aabb*. The *F*₁ hybrid is a double heterozygote with the genotype *AaBb*.

The *F*₂ plants with the prostrate growth habit (type I) as in *M. longiflora* are, thus, homozygous with regard to the gene *A* and homozygous or heterozygous with regard to the gene *B*, since the gene *b* would not manifest itself phenotypically in the presence of gene *B*.

The *F*₂ plants with the semi-prostrate growth habit (type II) have the gene *A*, the gene *a* manifesting itself phenotypically, and the gene *B* in the homozygous or heterozygous state.

The *F*₂ plants with the intermediate growth habit (type III) may have the genes *A* and *b* or the genes *a* and *B* in the homozygous state or they may be homozygous with regard to the gene *a* and heterozygous with regard to the gene *B*.

The plants with a growth habit similar to *M. jalapa* (type IV) have only one gene of the prostrate growth habit. It is not the gene *B* since owing to its being dominant over the gene *b* the effect would be the same as if there were two genes of the prostrate growth habit. These plants are heterozygous with regard to gene *A* and homozygous with regard to gene *b*.

The *F*₂ plants with the upright growth habit as in *M. jalapa* (type V) have genes *a* and *b* in the homozygous state.

The genotype formulae and relations corresponding to the classes of growth habits distinguished in F_2 are, therefore, the following:

- I. 1 *AABB*, 2 *AABb*
- II. 2 *AaBB*, 4 *AaBb*
- III. 1 *AAbb*, 1 *aaBB*, 2 *aaBb*
- IV. 2 *Aabb*
- V. 1 *aabb*

The other differences in the growth habit, such as the number of nodes, the length of internodes, and the number and the thickness of side stems, are not sufficiently distinct for a detailed analysis.

8. Morphology of leaves

The leaves of *M. jalapa* and *M. longiflora* are strikingly different. However, the difference results from the summing of many, more or less independent minor traits difficult to define.



Fig. 13. Protograph showing the shapes of the leaf base in *M. jalapa*, *M. longiflora*, F_1 , and F_2 . Top row: left *M. jalapa*, right *M. longiflora*, in the middle a leaf of the F_1 plant. Bottom row: leaves of F_2 plants

These differences are:

Mirabilis jalapa

1. Base of leaf obtuse.
2. Base of leaf usually oblique.
3. Venation pinnate usually asymmetrical at base.
4. The tapering of leaves not gradual, at first slow then sudden.
5. Leaf tip sharply acute.

Mirabilis longiflora

1. Base of leaf cordate.
2. Base of leaf symmetrical.
3. Venation pinnate usually symmetrical at base.
4. The tapering of leaves gradual.
5. Leaf tip not sharply acute.

There are, moreover, some differences in the size of leaves, the length of petioles, the length to width ratio, and the kind and the distribution of the pubescence. The last mentioned trait will be discussed further on (page 630).

The difference in the shape of the leaf base is rather pronounced (Fig. 13).

The F_1 plant has leaves with the base shaped intermediately between the parental species. The leaves have two lobes as in *M. longiflora*, but as is to be seen on the photograph they are different.

In F_2 five types of the leaf base have been discriminated:

1. Leaf base same as in *M. jalapa*.
2. Leaf base approaching *M. jalapa*.
3. Leaf base intermediate same as in F_1 .
4. Leaf base approaching *M. longiflora*.
5. Leaf base same as in *M. longiflora*.

Table 4

The inheritance of the shape of the leaf base

Class values	I	II	III	IV	V
M.j.	+				
M.l.					
F_1			1		
F_2	39	55	41	54	55

However, these classes have been discriminated artificially and probably the variation in F_2 actually is continuous.

The more accurate analysis of the manner in which the shape of the leaf base is inherited has been made impossible by the great phenotypical variation, so that in one plant there could be simultaneously leaves with the base shaped according to two, three, or even four of the discriminated types.

Moreover, in various years the same plant could be classified according to the shape of its leaf base to different, though not extremely different, classes, which proves how strongly the shape of the leaf base fluctuated.

The symmetry or the asymmetry of the leaf base is not related with its shape.

The manner in which the leaf blade tapers off is very difficult to define, but probably is not strictly related with the shape of the leaf base.

M. jalapa has larger leaves than *M. longiflora*. In ten *M. jalapa* plants the average length of the leaves from the seventh internode ranged

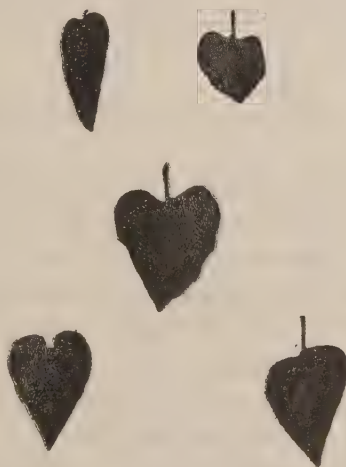


Fig. 14. Photograph showing the leaves in *M. jalapa* (top, left), *M. longiflora* (top right), F_1 (middle), and the extreme forms in F_2 (bottom row)

89 to 138 mm. The analogical averages in five *M. longiflora* plants ranged 65 to 83 mm. In the F_1 plant the average length of leaves was 81 mm. The corresponding values for the F_2 plants were 36 to 123 mm.

The petioles are much longer in *M. jalapa* than in *M. longiflora*. The average length of petioles in the former species was ± 20 mm. and in the latter ± 1 mm.

The F_1 plant has petioles of the same length as in *M. jalapa*, whereas in the F_2 generation there were plants with petioles as long as in *M. jalapa* and of the intermediate lengths, but no plants with petioles as short as in *M. longiflora*.

The leaves of *M. jalapa* and *M. longiflora* also differ in the width to length ratio.

In *M. jalapa* the ratio is somewhat lower and for the particular leaves varied within the range 0.450 to 0.750 and in *M. longiflora* within the range 0.650 to 0.900. The corresponding values in the F_1 plant were 0.650 to 0.800.

In the F_2 generation the variation is in a continuous series ranging from one parental species to the other. There are, moreover some plants with leaves much narrower than in *M. jalapa* and wider than in *M. longiflora*.

In the plants with the narrowest leaves the width to length ratio was 0.410 and in those with the widest leaves it was 1.071. Fig. 14 shows leaves of the F_2 plants with the narrowest and the widest leaves (bottom row), a leaf of the F_1 plant (middle row), and one leaf from each of the parental species (top row).

All the details here described show that the morphological differences between the leaves of the two species are very complex. The variations of these traits fluctuate very strongly and the traits are, therefore, unsuitable for a detailed analysis.

9. Pubescence on stems, leaves, and flowers

The degree and the kind of pubescence on the stems, the leaves, and the flowers in *M. jalapa* and *M. longiflora* are very different.

The stems of *M. jalapa* are covered with short, downy, almost appressed hairs. The distribution of the pubescence is not uniform. The hairs are mainly grouped in two strips stretching along the stem on its opposite sides.

The average length of hairs ranges 300 to 800 μ . In the dense strips of pubescence the distances between the hairs in the lengthwise rows usually is from 100 to 300 μ . The analogical distances in the parts with less dense hairs are 600 to 2000 μ .

The hairs in *M. jalapa* and the manner in which they grow on the stems are shown in fig. 15 a.

The stems of *M. longiflora* are covered by uniformly distributed, upright glandular hairs.

The length of these hairs is usually from 150 to 900 μ . The distances between the hairs in the lengthwise rows average at 100 to 500 μ .

Owing to the large number of the glandular hairs the stems of *M. longiflora* are distinctly sticky. Among the glandular hairs ending with the spherical secreting cell there are occasionally some non-glandular hairs ending with a long thin cell as in *M. jalapa* and other as if intermediate hairs ending with slightly swollen club-like cells.

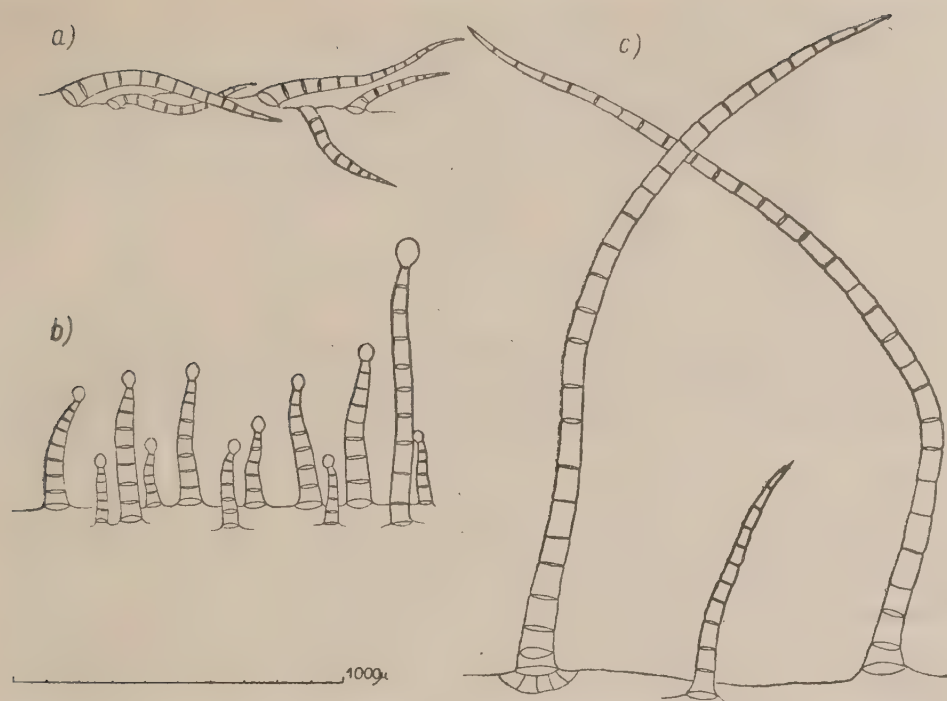


Fig. 15. The size, the shape, and the position of hairs on stems. *a* — *M. jalapa*; *b* — *M. longiflora*; *c* — One of the F_2 plants

The glandular hairs of *M. longiflora* and their arrangement on the stem is shown in fig. 15 b.

In the F_1 hybrid the distribution of pubescence on the stems is not uniform. Most of the hairs are grouped as in *M. jalapa* in two opposite strips. However, the difference between the density of hairs in the denser strips and the other parts of the stem is not as great as in *M. jalapa*. The hairs are not glandular but less appressed than in *M. jalapa*.

The length of hairs is usually from 150 to 800 μ . Some hairs are longer, up to 1200 μ long, and upright. Occasionally there are hairs with a swollen end cell.

In the F_2 hybrids the pubescence on the stems is very varied. There are individuals with hairs distributed as in *M. jalapa* and others with the hairs as uniformly distributed as in *M. longiflora*, as well as the full series of intermediate distributions. The pubescence could be appressed as in *M. jalapa* upright as in *M. longiflora*, and of many intermediate types. The cell at the end of hairs also are of various kinds: there are cells sharply tapering as in *M. jalapa* (Fig. 16 e), swollen and almost as spherical as the cells of the glandular hairs in *M. longiflora* (Fig. 16 a), or of all

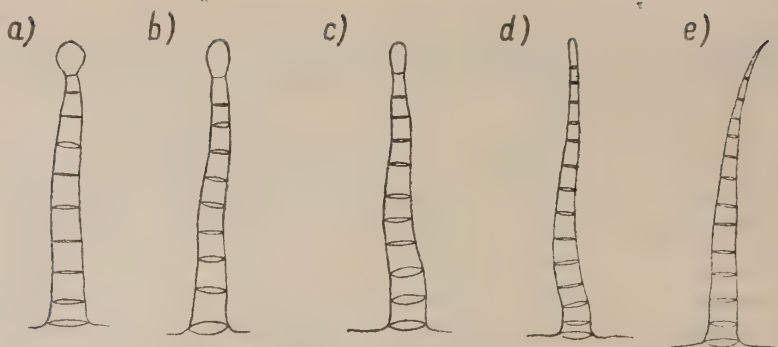


Fig. 16. Types of tip cells of hairs in the F_2 generation. *a* — Hair of type approaching *M. longiflora*; *b*, *c*, *d* — Intermediate hairs; *e* — Hair of type approaching *M. jalapa*

the intermediate forms (Fig. 16 *b, c, d*). The hairs of all these different types may occur side by side on the same plant.

However, even the hairs with the most rounded cells at the tip are not glandular, because the stems and other organs having this kind of pubescence are never sticky. This circumstance was already pointed out by *Prakken* (1944).

The absence of secreting cells at the tips of hairs leads to the conclusion that the type of pubescence characteristic for *M. longiflora* is not segregated in the F_2 generation, though among the 150 examined individuals there were 20 with hairs of a similar kind and similarly distributed as in *M. jalapa*. In 26 other individuals the stems were uniformly covered with hairs. Very often a stem was covered with two kinds of pubescence: upright bristly hairs covered more or less uniformly the whole stem, whereas the appressed, shorter, downy hairs were mainly grouped in two opposite strips.

In the plants of the F_2 generation the pubescence on the stems often was less dense and longer than in the parental species. An extreme example of this kind of pubescence was supplied by the plant in which the length of the longest hairs ranged 700 to 3000 μ . The longest hairs of this plant were about four times longer than the hairs in either *M. jalapa* or *M. longiflora* (Fig. 15 *c*).

The pubescence of leaves in both parental species shows similar differences concerning distribution, morphological characters of different kinds of hairs and position of hairs on the surface of the blade.

In F_1 the distribution of the hairs is similar to that in *M. jalapa* and other traits are more or less intermediate.

In F_2 a distinct segregation was observed but in any plant the type of

appressed hairs typical for *M. jalapa* was found. Also the glandular type of hairs of *M. longiflora* was not reconstructed.

The type of pubescence on floral tubes was in parental species, F_1 and F_2 hybrids more or less similar to that described for the stems. However, the pubescence of the calyx and its inheritance was almost the same as in the case of leaves.

10. Colour of stems

In *M. longiflora* the main and the secondary stems have green internodes and reddish nodes. The reddish colour of the nodes is caused by anthocyanin in the epidermal cells and also in some of the neighbouring collenchyma cells.

Anthocyanin present in the flowers of *M. longiflora* causes the pale pink-violet colour of the inside of the flower tubes and the red-violet ring at the point where the tube widens to form lobes.

The colour of the stems in *M. jalapa* differs in the particular varieties. The stems of the variety with white or yellow flowers are all green and contain no anthocyanin.

The stems of the variety with red or pink flowers have red nodes, usually more intensely red than the nodes in *M. longiflora*, and green often red-streaked internodes.

The hybrid of this investigation was obtained by crossing a *M. longiflora* plant with a *M. jalapa* plant of the red-flower variety. Thus, both parental plants have red nodes and green internodes.

The F_1 plant has stems with red nodes and distinctly pink-streaked internodes.

In the F_2 generation the plants with stems coloured in a similar way as in both parental plants predominated. However, there were also some plants with much more intensely coloured stems and others with stems coloured less intensely than in the parental plants.

Among 240 examined F_2 plants the following three degrees in the intensity of the red colour of stems were discriminated:

I. Plants with more or less the same colour of stems as in the parental plants $n = 118$.

II. Plants with stems less intensely coloured than in the parental plants, $n = 45$. This group included plants with:

- a) nodes and internodes all green, $n = 2$, and
- b) green-brown nodes and green internodes, $n = 43$.

III. Plants more intensely coloured than the parental plants, $n = 77$. This group included plants with:

- a) the whole stems intensely red, $n = 15$,

b) stems all pink, $n=12$, and

c) nodes and all internodes, except for the last three or four, red, $n=50$.

The great phenotypic variability of this trait has made its detailed analysis difficult and the manner in which the trait is inherited is not discussed here.

11. Colour of flowers

The flowers in *M. longiflora* are white, except for the inside of the flower tube, which according to the Horticultural Colour Charts is cyclamen purple 30, and the ring the colour of which is orchid purple 31.

M. jalapa has flowers of different colours — yellow, red, or white.

The progeny obtained by crossing the varieties with differently coloured flowers often have flowers of very peculiar red or orange-red tones.

The *M. jalapa* plant used as the parental plant for the F_1 generation had red — magenta red 27 — flowers with the inside of the tube and the ring somewhat more violet in tone — i.e. rhodamine purple 29.

The colour of the flowers in the F_1 plant is mauve 633 with the inside of the tube and the ring cyclamen purple 30.

The segregation in F_2 was highly complicated. In all flowers independently of the colour of the lobes the colour of the inside of the tube and of the ring was cyclamen purple, rhodamine purple, or orchid purple.

The colours of the lobes were of different tones and different intensities.

An additional difficulty in the classification of flower colours was that in about 20 per cent of the F_2 plants the colour at the base of lobes was of a different shade than in the upper part of the lobes.

For the sake of simplicity in the descriptions of the colour of the lobes the difference in colour at the lobe base is here disregarded.

Among 153 examined F_2 plants not one with white lobes as in *M. longiflora* was found.

In only one plant the colour of the lobes was magenta 27, i.e. the same as in the parental *M. jalapa* plant.

The conclusion to be drawn from this result is that the colour of lobes is determined by more than two pairs of genes.

About 15/16 of the plants in the F_2 generation had violet-pink flowers with various proportions between these two colours. The flowers ranged from very distinctly violet as in the F_1 plant to almost pure pink or red with only small additions of violet tones. All these colours were of various intensities ranging from almost white to very dark.

About 1/16 of the F_2 plants had flowers of various colours without any violet tones. There were ten such plants and their colours were as follows:

magenta 27 (red)	1 plant
magenta rose 027 (dirty red)	6 plants
jasper red 18 (red-orange)	2 plants
claret rose 21	1 plant
crimson (warmer red than magenta)	1 plant

The occurrence of these colours in the F_2 generation can be explained by assuming as did Marryat (1909), that the colour of the lobes in *M. jalapa* is determined by two pairs of genes.

According to this supposition the *M. jalapa* plants with red flowers are heterozygous or homozygous with regard to the gene *R* responsible for the red colour and homozygous or heterozygous with regard to the gene *Y* responsible for the yellow colour. The red colour cannot appear without at least one gene *Y*, so that even when there are two *R* genes the colour of the flower is white as long as a *Y* gene is missing.

According to Marryat the genotype of *M. jalapa* can be as follows:

when the colour of flowers is magenta the genotype is $Y y R R$,

when the colour of flowers is crimson the genotype is $Y Y R R$,

when the colour of flowers is various shades of orange-red the genotype is $Y Y R r$,

when the colour of flowers is magenta rose the genotype is $Y y R r$.

The presence in the F_2 generation of plants with flowers of these colours indicates that the original F_1 plant must have in its genotype the gene *Y* of *M. jalapa*.

If the genotype of *M. longiflora* only has the same genes as the white variety of *M. jalapa*, then the above-mentioned types would have to be much more numerous and there also would have to be a large proportion of plants with yellow and white flowers. This would also leave unexplained the presence of violet tones not occurring in *M. jalapa*.

This circumstance makes it necessary to accept Prakken's assumption that *M. longiflora* has genes determining the violet colour of petals which cannot manifest themselves owing to the absence of colour intensity factors present in the genotype of *M. jalapa*.

The fact that all the plants without any violet tones in the colour of their flowers only amounted to 1/16 of the whole F_2 population leads to the conclusion that in the genotype of *M. longiflora* there are at least two independent pairs of genes $V_1 V_1$ and $V_2 V_2$, which confirms Prakken's supposition. These genes are not allelomorphic with regard to the previously mentioned genes of *M. jalapa*.

According to these assumptions the parental plants probably have the following genotypes:

M. jalapa
Y y R R v₁ v₁ v₂ v₂

M. longiflora
y y r r V₁ V₁ V₂ V₂

Moreover, the genotype of *M. jalapa* must contain various colour intensity factors I₁ I₁ I₂ I₂ I₃ I₃. The presumable genotype of the F₁ plant is Y y R r V₁ v₁ V₂ v₂, whereas the 1/16 of the F₂ plants without the violet tones in the flowers do not contain the V₁ and V₂ genes and have various combinations of the Y and R factors.

Probably there are many other genes responsible for the different shades of the lobe base or of the apical parts of the lobes and possible determining the lack of uniformity in the distribution of various pigments in the lobes. However, a detailed analysis of these factors would be extremely complicated and has not been considered.

12. Date of flowering

Under optimum conditions the two *Mirabilis* species of this investigation begin to flower almost simultaneously.

In various years *M. jalapa* begins to flower between the middle of July and the first days of August.

In 1959 ten *M. jalapa* plants flowered between July 17 and 23. In the much less sunny summer of 1960 these plants began flowering between July 31 and August 8.

In 1960 five plants of *M. longiflora* began flowering between August 5 and 10.

However, the difference in the date of flowering between the two species is so small only when the insolation is sufficient. *M. longiflora* is much more sensitive to the intensity of light.

The usual difference in the flowering date between the two species when they grow on a well shaded stand is about one month. Sometimes the *M. longiflora* plants do not flower at all, whereas the delay in the flowering of *M. jalapa* is only about two weeks.

In the further comparisons of the flowering dates only the optimum conditions have been considered.

The F₁ plant flowers approximately on the same date as *M. jalapa* and *M. longiflora*.

In 1960 the F₁ plant flowered on July 29. In the same year the first F₂ plants flowered on July 19 and the last on September 22.

Table 5

Dates at which the plants flowered

Class values	19 VII	25 VII	31 VII	6 VIII	12 VIII	18 VIII	24 VIII	30 VIII	5 IX	11 IX	17 IX	23 IX	N
M.j.			9	1									10
M.l.			1	4									5
F ₁		1											1
F ₂	5	9	14	41	38	15	9	3	6	3	9		152

Table 5 lists the number of plants flowering at different dates.

This table shows very clearly the great differences in the beginning of the flowering of various plants. These differences would be even greater if on the night of September 24 the temperature did not drop below -1°C so that most of the plants were frosted. In this way ten F₂ plants did not flower at all.

So great differences in the date of flowering of F₂ plants can not only be due to the large number of plants in this generation as compared to the numbers of the parental species.

13. Time of anthesis

The genus *Mirabilis* has one-day flowers opening in the afternoon and withering the same night.

Under the same environmental conditions the time of anthesis is different in the various species and varieties.

On any particular day the time of anthesis is influenced by various factors, such as the temperature, the insolation, air humidity etc. The influence of temperature on the time of anthesis is the strongest.

On cold days at the end of the summer the flowers open much later and in plants which even on warm days flower late in the day on cold days they often do not open at all.

On heavily clouded days the rate of anthesis also is retarded but clouds never stop the opening of flowers.

The flowers of *M. longiflora* open between 4^h15' and 5^h30' p.m. depending on the prevailing weather conditions.

The flowers of the white variety of *M. jalapa* open between 6 and 7^h30' p.m. and of the red variety even later, between 6^h30' and 8 p.m. (sometimes they do not open at all).

The flowers of the F₁ plant open between 5^h30' and 6^h30' p.m.

On any given day the differences between the time of anthesis in the

particular plants of *M. longiflora* or in the particular plants of the *M. jalapa* varieties are insignificant.

On the other hand, the time of anthesis in the particular plants of the F_2 generation on any given day differ strikingly. In the plants flowering earliest in the day the flowers open approximately at the same time as in the parental plant opening its flowers earliest, i.e. at the same time as *M. longiflora*. The last F_2 plants flower somewhat later than the late flowering parent, i.e. later than *M. jalapa*.

Observations indicate that the hour of anthesis in any given F_2 plant is not accidental. The F_2 plants flowering earliest on some one day also flower earliest on any other day and, similarly, the plants in which the flowers on one day open at an intermediate or late hour also flower at an intermediate or late hour on all other days.

Although the anthesis time of a particular plant is different on different days, there is a rather well established sequence in the flowering of the particular plants of the F_2 generation which persists from day to day.



Fig. 17. Relation between the times of anthesis in F_2 plants on two different days

Since the time of the day at which the flowers open depends on many factors, the differences in the sequence of the opening of flowers among the plants of the F_2 generation increase with any increase of the number of factors by which one day differs from another.

Fig. 17 shows the relationship between the recorded times of anthesis of F_2 plants on two days with a similar insolation which differed markedly only in the prevailing temperatures. The maximum temperature was 26°C on September 20 and 23°C on September 21. The points on the graph in fig. 17 represent the particular plants. The position of the points with regard to the x-axis corresponds to the time of the day at which the flowers of a plant opened on September 20 and their position with regard to the y-axis corresponds to the time on which the flowers opened on September 21. The distribution of the points on the graph is ordered which proves that the time of the day at which the plants of the F_2 generation flowered was not accidental.

The differences in the time of anthesis might have been caused by various environmental conditions in the different parts of the experimental plot. However, this does not seem to be the case, since in seven *M. jalapa* plants of the red variety distributed at random over the whole field among the F_2 plants the flowers opened almost simultaneously.

It seems, therefore, that the time of anthesis is determined genetically and that this trait is inherited in a similar way as any other quantitative trait.

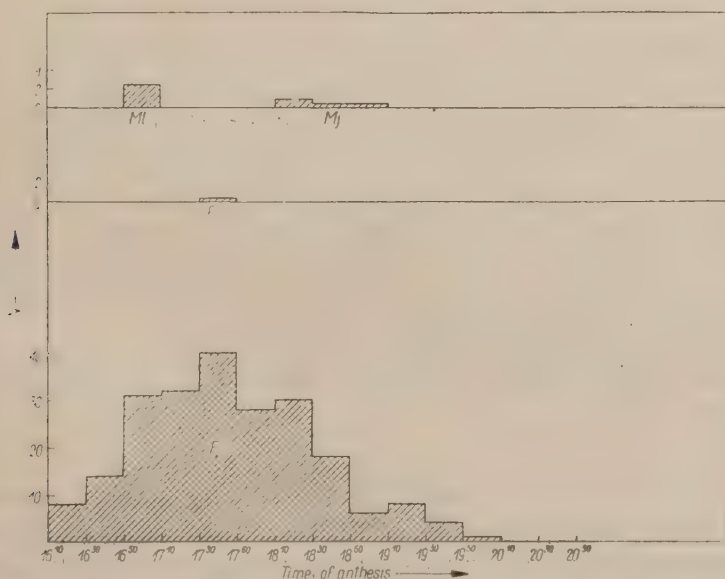


Fig. 18. Frequency histogram of the average time of the day at which anthesis occurs in *M. jalapa*, *M. longiflora*, F_1 , and F_2 .

The numbers of plants flowering at intervals of ten minutes on September 20, 1960, are shown in fig. 18. As is to be seen the frequency distribution in this graph is essentially similar to the other graphs illustrating the distribution of the other quantitative traits.

14. Pollen fertility

The pollen fertility in *M. jalapa* is normal ranging in the plants from 87 to 96 per cent, average 91 per cent.

In *M. longiflora* the pollen fertility is reduced and ranges in the five examined plants between 64 and 87 per cent, average 71 per cent.

The pollen fertility in the F_1 plant is 16 per cent, whereas in 195 examined F_2 plants it ranges between 1 and 82 per cent. The results of the pollen fertility counts are listed in Table 6.

Table 6
Pollen fertility in percents

Class values	0	10	20	30	40	50	60	70	80	90	100	N
M.j.									6	4		10
M.l.								3	1	1		5
F_1			1									1
F_2	28	61	42	20	20	11	4	8	1	0		195

The greatest number of F_2 plants are in the same class of pollen fertility as the F_1 plant. In 54 per cent of F_2 plants pollen fertility was higher than in the F_1 plant and only in 14 per cent of plants pollen fertility was lower. Only in one plant, i.e. in about 0.5 per cent of the F_2 population, pollen fertility was more than 80 per cent.

The pollen fertility counts were very difficult because of the great variability of this trait in the particular flowers of a plant with similarly developed and similarly coloured anthers. Owing to this large number of pollen grains had to be counted in each plant.

Moreover, pollen fertility of a given plant differed considerably from year to year.

DISCUSSION

In the course of this investigation disturbances of the reduction divisions were found in the PMCs of *M. jalapa*. This result contradicts Prakken's report about the regular course of meiosis in both *M. jalapa* and *M. longiflora*.

However, it seems that in the F_1 plant disturbances of meiosis are more frequent than in *M. jalapa*. This again contradicts the results obtained by Showalter who found that the percentage of disturbed meioses in the F_1 hybrid was approximately the same as in the parental species.

The rather frequent disturbances of microsporogenesis in the F_1 plant are however insufficient to explain the very low pollen fertility in this plant. It seems that besides the visible chromosome disturbances the pollen sterility must also be caused by gene sterility or cryptic structural hybridity.

One point already emphasized by Showalter must be stressed here. The anthers in the flowers of the F_1 hybrid produce much less pollen than in the parental species, owing to the degeneration of PMCs beginning before meiosis, which must be caused by gene sterility.

In the F_2 generation there is a certain increase in the pollen fertility as compared to F_1 , which is reflected by that 54 per cent of the F_2 plants have a pollen fertility higher than in F_1 and only in 14 per cent of plants pollen fertility is lower than in F_1 .

The higher fertility of gametes in F_2 is a rather frequent phenomenon. It is reported by e.g. Clausen in the genus *Viola* (1926, 1930). Müntzing in the cross *Galeopsis tetrahit* × *G. bifida* (1930), and Anderson in the cross *Nicotiana langsdorffii* × *N. alata* (1936). According to those authors the higher pollen fertility in F_2 is caused by the segregation of some factors responsible for the sterility of gametes in the heterozygous condition only.

However, as compared with the parental species the pollen fertility of the F_2 plants is very much reduced.

The very low setting of seeds in the F_1 and F_2 generations does not seem to be caused by the reduced pollen fertility, though this often is the case in other interspecific hybrids, e.g. in the cross *Aquilegia vulgaris* × *A. ecalcarata* (Pražmo 1960). Out of nine plants in the F_2 generation with pollen fertility of more than 70 per cent five did not set seeds at all and four developed one to five seeds.

The plant on which 34 well developed seeds were collected — this was the highest number of well developed seeds collected on any one of the F_2 plants — only had 24 per cent of good pollen grains. Unfortunately, owing to great technical difficulties the exact correlation between the setting of seeds and pollen fertility has not been calculated.

There are very good reasons to suppose that the factors determining the length of styles are to some extent independent of the factors responsible for the length of the pollen tube. If this was the case, a certain number of F_2 plants with very long styles would produce disproportionately short pollen tubes. Then the failure in the setting of seeds in such plants

would be primarily caused by the pollen tubes not reaching to the ovules, thus making fertilization impossible. This probably is the reason why all attempts at the cross *M. longiflora* ♀ × *M. jalapa* ♂ always fail. However, this does not seem to be of significance, since on the whole the F_2 plants with short styles do not set more seeds than the plants with long styles, as they would if the low rate in the setting of seeds in the F_2 generation was primarily caused by the too short pollen tubes.

A low seed fertility in the F_1 and F_2 generations is very common in similar interspecific crosses. The hybrid *M. jalapa* × *M. longiflora* resembles under many regards other hybrids of this kind.

Very characteristic is the polymerous segregation of quantitative traits — e.g. the flower tube length, the style length, the length and the width of lobes etc. — and the Mendelian segregation of other traits — e.g. the colour of flowers, the growth habit. However, the inheritance of the flower tube length is rather unusual. Although in F_2 the variation of the tube length has the normal frequency distribution the curve is shifted towards one of the parental species so that the variation in F_2 includes the variation in *M. jalapa* but does not extend to *M. longiflora*.

This deviates from the normal variation for which there usually are only two alternatives: either in the F_2 generation there are in approximately equal numbers individuals having the particular quantitative trait developed in the extreme forms as in the parental species (e.g. plants with flower tubes as short as in *M. jalapa* or as long as in *M. longiflora*), or the F_2 plants resemble with regard to the trait in question neither parental species.

Inheritance is according to the latter alternative when a given trait is determined by a very large number of polymerous factors. Then the probability that individuals with the trait expressed in the extreme form will appear is so small as to become practically an impossibility.

In experiments with the genus *Nicotiana* Smith (1937) crossed species which differed, similarly as the *Mirabilis* species, in the length of flower tubes. The average length of the short tubes in *Nicotiana langsdorffii* was about 18 mm., whereas the average length of the much longer tubes in *Nicotiana Sanderae* is about 70 mm. In the F_1 generation the length of the flower tubes was intermediate and in the F_2 generation the variation exceeded the variation in F_1 , but extended to neither of the two possibilities represented by the parental species. The length of the flower tubes was 23 mm. in the F_2 plant with the shortest tubes and 55 mm. in the plant with the longest tubes. This is the simplest example of inheritance dependent on a large number of polymerous genes.

The supposition may be advanced that, in view of the very low

pollen and seed fertility in the F_1 plant, the unusual manner in which the length of the flower tube was inherited in the cross *M. jalapa* $\times$ *M. longiflora* is caused by that some genotypes are not eliminated at random. However, the improbability of this kind of elimination is demonstrated by the normal segregation of most other traits in the F_2 generation, in particular by the almost ideal segregation of the various types of the growth habit.

The absence in the F_2 generation of the cross *M. jalapa* $\times$ *M. longiflora* of plants with long flower tubes of the *M. longiflora* type may have various causes.

One highly probable explanation is that it is simply the result of cytoplasmic heredity, since *M. jalapa* was here the maternal plant. Unfortunately the supposition can only be checked by measurements of the flower tube lengths in the second generation of the reciprocal cross. This, however, is very difficult, if not impossible, since in spite of repeated attempts by many workers such crosses have never been successful.

This explanation for the abnormal variation of the flower tube length in the F_2 generation has been advanced by Prækken who also thought it highly probable that the absence of plants with long flower tubes of extreme lengths was caused by the large number of polymerous factors determining this trait. However, this opinion does not seem to be justified, since when the numerous polymerous factors are assumed it is impossible to explain the presence of plants with the short flower tubes similar to the flower tubes in *M. jalapa*.

The most plausible explanation of this phenomenon seems to be the presence in the genotype of *Mirabilis jalapa* of inhibitors counteracting the factors responsible for the elongation of the flower tube in *M. longiflora*.

Let us assume that the difference between the length of flower tubes in *M. jalapa* and in *M. longiflora* is determined by a certain number of independent loci and let there be three such loci.

Let us assume, moreover, that *M. jalapa* is homozygous with regard to the genes $l_1 l_2 l_3$, whereas *M. longiflora* is homozygous with regard to their alleles $L_1 L_2 L_3$.

The genes represented by the small letters have no influence on the lengthening of the flower tube, whereas each of the alleles represented by the capitals increases the length of the flower tube adding to it some fixed amount or multiplying it by some fixed factor.

Besides the genes just mentioned let us assume that in the genotype of *M. jalapa* there are the inhibitors $I_1 I_2 I_3$. Then the genotype of *M. jalapa* would be $l_1 l_1 l_2 l_2 l_3 l_3 I_1 I_1 I_2 I_2 I_3 I_3$ and the genotype of *M. longiflora* $L_1 L_1 L_2 L_2 L_3 L_3 i_1 i_1 i_2 i_2 i_3 i_3$.

Finally let us assume that when *M. jalapa* is crossed with *M. longiflora* each of the inhibitor genes acts so as to prevent the manifestation of the effects of any of the two pairs of genes L_1 and L_2 . Each of the inhibitors can also inhibit all these four genes simultaneously. However, the inhibitors do not affect the third pair of genes L_3 l_3 . The genotype of the F_1 generation would then be $L_1 l_1 L_2 l_2 L_3 l_3 I_1 i_1 I_2 i_2 I_3 i_3$.

Thus the flower tubes in the F_1 generation would be longer than the average tubes in *M. jalapa* and shorter than the arithmetic mean or the geometric mean length of tubes in the two parental species.

The plants with flower tubes as short as in *M. jalapa* would then constitute $\frac{1009}{4096}$ of all the plants in the F_2 generation. These would be those plants homozygous for l_3 do not have at the same time any L_1 or L_2 genes together with i_1, i_2, i_3 in homozygous condition.

The plants with flower tubes as long as in *M. longiflora* can have none of the $l_1 l_2 l_3$ and of the $I_1 I_2 I_3$ genes and, according to the probability for six segregating genes, would constitute $1/4096$ of the whole F_2 population. The probability that such a plant would appear in a population of the size obtained in this investigation is extremely small.

The probability of the occurrence in F_2 of plants with flower tubes much longer than in F_1 also is very small.

The plants with the longest flower tubes that can actually be found in the F_2 generation can have tubes not much longer than the tubes in F_1 . Such plants would be homozygous with regard to the gene L_3 which is not affected by the inhibitors.

The model here described undoubtedly greatly simplifies the actually existing conditions, but it may reflect them more or less accurately.

In many interspecific and intervarietal crosses the presence in one group of genes inhibiting the effect of genes from the other group has often been observed. Such inhibitors were found e.g. by Clausen and Hiesey (1958) in crosses between two subspecies of *Potentilla glandulosa*. One of the subspecies used for the cross had incised petals, whereas the petals in the other subspecies were entire in the usual way. On the ground of an analysis of the F_1 generation and of the segregation in F_2 and F_3 Clausen and Hiesey reached the conclusion that this trait was controlled by at least three independent pairs of genes: one pair of genes NN causing incision and two pairs of incision inhibitors $I_1 I_1$ and $I_2 I_2$.

The plant with incised petals used for the cross contained no inhibitor genes and was homozygous with regard to the incision gene N , which in the absence of the inhibitors was dominant. The plant with entire petals contained no incision genes and was homozygous with regard to the genes I_1 and I_2 .

In the cross described by Clausen and Hiesey the inhibition of the incision gene had different intensities according to the number of the inhibitors present in the genotype.

In the model here described the situation is different. The effect of one inhibitor is as strong as of all of them acting together.

There can also be other reasons for the untypical segregation of the flower tube length in the plants of the F_2 generation of the cross *M. jalapa* × *M. longiflora*.

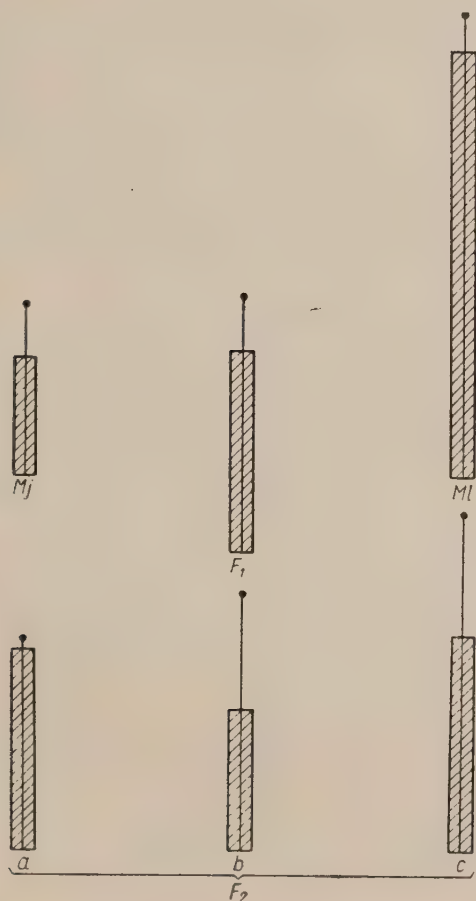


Fig. 19. Examples of plants having flowers with unadjusted flower tube and style lengths. *a* — Schematic diagram of a flower in a F_2 plant with the style too short as compared to the length of the flower tube; *b, c* — Schematic diagrams of flowers in F_2 plants with styles too long as compared to the length of the flower tubes. The flowers of *M. jalapa*, *M. longiflora* (top row), and the F_1 plant (middle row) are shown for comparison.

Smith's (1937) work on the influence of an additional chromosome on the size of the corolla in *Nicotiana* has demonstrated that in the genotype of the species with the short flower tube there are also "factors elongating the tube", whereas in the genotype of the species with long flower tubes there are "factors of the short tube length".

The assumption that in the genotype of *M. longiflora* there are certain factors "of the short tube length" as well as recessive inhibitors corresponding to these factors in the homozygous state also explains the unusual segregation in F_2 .

Another observation made in this investigation on the cross *M. jalapa* $\times$ *M. longiflora* is that, in spite of the correlation between the lengths of the flower tube and the style clearly marked for the F_2 generation as a whole, there also are in this generation single plants in which the length of the style is as if unadjusted to the length of the tube (Fig. 19).

In some instances there is a certain "unadjustment" between the length of the style and the length of stamens. In both parental species stigma is on average 2 to 10 mm above the anthers. However, in F_2 there are plants which have anthers on the same level as the stigma and even in a few plants the anthers are above the stigma.

The circumstance that such a physiological trait as the time of the day at which anthesis takes place is determined genetically also seems of significance. The genetical determination of this trait is indicated by the constant and approximately uniform differences in the time at which the flowers open in *M. jalapa* and *M. longiflora* as well as the essentially unchanging order in which anthesis occurs in the particular plants of the F_2 generation. The segregation with regard to this physiological trait is polymerous, similarly as in the case of other quantitative traits.

The stability of the time of the day at which the flowers open in the various *Mirabilis* species has long attracted attention and this peculiarity is even reflected in their popular name four o'clock.

This investigation on the heredity of the specific traits in the cross *M. jalapa* $\times$ *M. longiflora* has shown that, in spite of the great morphological differences between the two species, especially striking in the case of the size of flowers, a genetic analysis is possible. The results reported here may serve as a contribution to the studies on the genetic principles underlying interspecific differences and on the speciation processes.

CONCLUSIONS

1. The haploidal chromosome number in the species *Mirabilis jalapa* and *M. longiflora* is $n = 29$.

Numerous PMCs were examined in *M. jalapa* and a few univalents were found.

2. In metaphase I of the hybrid *M. jalapa* × *M. longiflora* there are usually one or two pairs of univalents, though often there are 29 bivalents. Usually the two largest chromosomes of the *Mirabilis* karyotype fail to conjugate with each other.

3. The strongly reduced pollen fertility in the F_1 hybrid is about 16 per cent. The setting of seeds is also greatly reduced.

4. In spite of the very low fertility of the F_1 hybrid most of the traits characteristic for the parental species segregate regularly in F_2 .

5. The majority of the investigated morphological traits — such as the lengths of the flower tube, the style, the lobes of the corolla and of the calyx, as well as the widths of the lobes — manifest a continuous, sometimes transgressive variation caused by the segregation of numerous polymerous factors.

6. The length of the style and the length of the flower tube are inherited in a way giving a continuous variation in the F_2 generation. The range of the variation includes *M. jalapa*, but does not extend to the variation of the other parental species only slightly exceeding on the side of *M. longiflora* the variation in F_1 .

The pubescence is inherited in a similar manner.

7. One of the physiological traits — the time of the day at which anthesis occurs — also manifests a continuous variation in F_2 . This proves that the trait is controlled by numerous polymerous factors.

8. Such traits as the colour of flowers and the growth habit of stems manifest the typical Mendelian segregation.

This investigation was carried out at the Department of Genetics of the Warsaw University under the guidance of Professor W. Gajewski to whom I am greatly indebted for the help and valuable advice generously offered through all the stages of this work. My very special gratitude is also due to A. Doroszewska M. Sc. who supplied the one F_1 hybrid she had obtained and part of the seeds for the F_2 generation.

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Zmienność kwiatów *Parnassia palustris* L. w zależności od wysokości nad poziom morza

Variability of the flowers of Parnassia palustris L. depending on the height
above sea-level

ZOFIA RADWAŃSKA-PARYSKA

WSTĘP

Parnassia palustris L. — dziewięciornik błotny — jest rośliną dość rozpowszechnioną, w niektórych okolicach obficie występującą, która od dawna już wzbudzała zainteresowanie badaczy, przede wszystkim dzięki swym osobliwym i rzucającym się w oczy frędzelkowatym prątniczkom (staminodiom).

Od Sprengla — który w 1793 opisał biologię kwiatów dziewięciornika — ciągnie się długi szereg nazwisk tych, którzy aż po ostatnie czasy interesowali się biologią tego skromnego i dość pospolitego kwiatu.

Kwiat dziewięciornika, na pozór promienisty, wykazuje przy bliższym zbadaniu słabą grzbiecistość (zygomorfizm), na co już dawniej zwrócono uwagę. Goebel (1924) nazywa dziewięciornik kwiatem skryto-grzbieto-brzusznym (kryptodorsiwentralny); tę samą cechę podkreślają również Braun-Blanquet (ap. Hegi), Martens (1936) i Kozo-Polanski (1947).

Na ogół kwiaty dziewięciornika są bardzo prawidłowo rozwinięte i wykazują tylko nieliczne anormalności. Wśród zbadanych przeze mnie ponad 2500 egzemplarzy spotkałam zaledwie dwa razy kwiat o 6 płatkach, dwukrotnie kwiaty o 6 płatkach i 6 działkach oraz jeden raz kwiat o 6 płatkach i 6 pręcikach.

Celem niniejszej pracy było zbadanie, czy i w jakim stopniu zmieniają się wymiary koron kwiatowych (średnice) dziewięciornika w zależności od wzniesienia jego naturalnych stanowisk nad poziom morza oraz czy równocześnie zmieniają się prątniczki.

ZASIĘG PIONOWY I WYSTĘPOWANIE

Według Kotuli (1889—1890) górna granica występowania *Parnassia palustris* w Tatrach sięga po 2123 m na szczycie Krzesanicy (K o t. „2124“ — wg starych pomiarów), ale według danych Pawłowskiego, Sokółowskiego i Wallischa (1928) granica ta sięga 2140 m na Mięguszwieckim Szczycie nad Czarnym. Jest to, jak dotąd, najwyższe znane stanowisko dziewięciornika w Tatrach.

Po stronie południowej Tatr Zachodnich i Bielskich — jak podkreśla Kotula (l. c.) — *Parnassia* występuje znacznie mniej licznie niż po północnej. Również Sagorski i Schneider (1888) wymieniają ją ze wspomnianej części Tatr jako tylko miernie częstą. Natomiast według moich własnych obserwacji *Parnassia* rośnie w południowej części Tatr Bielskich bardzo obficie, zwłaszcza w okolicy Tatrzańskiej Kotliny, Fajkowej, Jatek i Kopy Bielskiej.

Po stronie północnej całego łańcucha Tatr roślina ta jest bardzo częsta i przeważnie występuje — podobnie jak i u stóp Tatr — w obfitych skupieniach, choć na stosunkowo niewielkich obszarach. Występowanie jest związane z rodzajem podłoża: skały osadowe, jak wapień, dolomit, piaskowce, flisz, bardziej odpowiadają dziewięciornikowi, co podkreśla również Dostał (1950), bywają jednak stanowiska i na granicie, np. podawane przez Pawłowskiego (l. c.) z okolic nad Morskim Okiem: „po miejscach trawiastych rozpowszechniony choć nie częsty; najwyższej: Mięguszwiecki Wielki 1700, Cubryna 1710, Niżnie Rysy 1850, Mięguszwiecki nad Czarnym 2140“.

Dziewięciornik na granicie z północnych stoków Tatr znalazłam jeszcze poniżej Morskiego Oka, nad Rybim Potokiem w kosówce i trawach; ponadto w Dolinie Jaworowej w jej części dolnej i środkowej oraz wzdłuż Doliny Białej Wody.

Według zdania Kotuli (l. c.) *Parnassia* nie występuje po stronie południowej Tatr Wysokich. Stwierdziłam tam jednak istnienie przynajmniej dwóch jej stanowisk: jednego w Dolinie Kiezmarskiej przy tzw. Zimnej Studni (16 stanowisko w spisie), i drugiego — wskazanego mi przez L. O d l o ž i l i k o v ą — rozrzuconego w kilku miejscach w Dolinie Wielickiej. Największe skupienie dziewięciornika znajduje się tam przy ścieżce na Polski Grzebień, pod Mokłą Wantą (26 stanowisko w spisie), na wysokości od 1720 do 1750 m; *Parnassia* występuje tam w charakterze rośliny szczelinowej w pęknięciach skalnych oraz na małych trawiastych gzymsikach i półczkach zwilżanych płynącą wodą, wśród ziołorośli.

Pozostałe miejsca w tejże Dolinie Wielickiej to trzy małe stanowiska pod ścianami Ponad Ogród Turni i dwa, również niewielkie, pod ścianą Granatów Wielickich. *Parnassia* występowała tam w małych ilościach: po kilka lub najwyżej kilkanaście okazów na każdym miejscu.

Stanowiska w Dolinach Kiezmarskiej i Wielickiej byłyby więc pierwszymi znanymi stanowiskami dziewięciornika w Tatrach Wysokich po ich południowej stronie. ¹

Na Podhalu, u stóp Tatr, *Parnassia* występuje dość nierównomiernie: są miejsca, gdzie zarasta całe łąki, np. w okolicy Bukowiny Tatrzańskiej, gdzie indziej natomiast w ogóle nie można się jej doszukać, np. w niektórych okolicach Kotliny Nowotarskiej.

Charakterystyczne, że *Parnassia* — na niżu roślina wilgotnych łąk — w górach rośnie zwykle na miejscach niezbyt wilgotnych, często na otwartych, nasłonecznionych zboczach. Schroeter (1926) przypisuje to większej wilgotności łąk alpejskich, powodującej inną gospodarkę wodną roślin górskich w porównaniu z niżowymi. Jak sądzę, również większa ilość opadów, częste mgły i mżawki oraz większa wilgotność względna odgrywają w tej sprawie także niepoślednią rolę.

BIOMETRIA KWIATÓW

Punktem wyjścia podjętych badań było przypuszczenie, wyrażone przez Schroetera (l. c), że *Parnassia palustris* jest rośliną, której kwiaty mają się zmniejszać wraz ze zwiększaniem się wysokości stanowisk nad poziom morza. Zjawisko takie byłoby czymś wyjątkowym, gdyż jest to znany fakt, iż kwiaty roślin w górach zwiększają się w miarę wzrostu wzniesienia, przy jednoczesnym zmniejszaniu się części wegetatywnych danej rośliny.

Praca niniejsza miała przynieść potwierdzenie lub zaprzeczenie przypuszczenia Schrötera.

Kwiaty dziewięciornika zbierałam i prowadziłam obserwacje nad nimi w sezonach letnich 1952, 1953 i 1954 na obszarze całych Tatr. Dla celów porównawczych kwiaty takie zostały też zebrane i pomierzone z kilku stanowisk niżowych. Za przesłanie materiału z niżu dziękuję serdecznie mgrowi R. Bohrowi, p. J. Falińskiemu, mgrowi J. Kadłubowskiej, doc. dr K. Lubliner-Mianowskiej i prof. drowi B. Pawłowskiemu.

Ogólna ilość pomierzonych przeze mnie kwiatów *Parnassia palustris* wyniosła 2699 egzemplarzy. Kwiaty te pochodziły łącznie z 34 stanowisk. Stanowiska w Tatrach wybierałam na różnych wysokościach, od podnóża masywu górskiego aż po mniej więcej górną granicę zasięgu pionowego dziewięciornika, tj. od wysokości 850 m po około 2100 m n.p.m.

Z każdego stanowiska brałam rośliny bez wyboru, kolejno jak rosły, starając się zebrać — o ile to było możliwe — co najmniej 50 sztuk, aby przeciętna pomiarów mogła być jak najbardziej zbliżona do rzeczywistości. Niestety, nie wszędzie udawało się to osiągnąć, niekiedy bowiem kwiatów było zbyt mało. Tam gdzie *Parnassia* jest rzadkością, np. w Dolinie Wielickiej, w Dolinie Kiezmarskiej i in., pomiarów dokonywałam w tere-

nie na okazach rosnących. Każdoroczny okres zbierania materiału ciągnął się mniej więcej od połowy lipca do połowy września.

Pomiarów biometrycznych dokonywałam częściowo w terenie na roślinach rosnących, przeważnie zaś na zebranych materiale żywym, na świeżo, wkrótce po jego zebraniu, najdalej po upływie paru godzin. W tym ostatnim przypadku kwiaty były przechowywane w woreczkach nylonowych lub igelitowych. Sposób ten zapewnia utrzymanie roślin w stanie zupełnej świeżości do 48 godzin i więcej.

Średnice koron kwiatowych mierzone były przy użyciu podziałki milimetrowej. Każda korona mierzona była trzykrotnie, w trzech różnych kierunkach wzdłuż średnicy. Z trzech w ten sposób otrzymanych liczb brana była średnia arytmetyczna. Ułamki milimetra zaokrąglalam do całości dodając je lub odejmując w zależności od tego, czy wypadły powyżej czy poniżej pięciu dziesiątych. Różnice średnic pojedynczego kwiatu w takim trzykrotnym pomiarze zazwyczaj były nieduże: od 0,5 mm do 1 mm, bardzo rzadko do 2 mm.

Łącznie dokonałam ponad 8 tysięcy pomiarów. Okazów uszkodzonych o brakujących, opadniętych lub nadjedzonych przez owady płatkach nie mierzyłam, jak również i bardzo młodych, dopiero rozwijających się z pączka kwiatów.

Charakterystyka stanowisk

Poniżej podaję krótką charakterystykę poszczególnych stanowisk, na których została zebrana *Parnassia palustris* do pomiarów biometrycznych.

Stanowisko 1. Około 1,5 km na wschód od Redy (nad Zatoką Gdańską), około 10–20 m n.p.m. Torfiaste łąki. Spośród przesłanych 170 okazów było zdalnych do pomiarzenia tylko 60. 6.IX.1954.

Stanowisko 2. Nad jeziorem Płaczewo, pow. gdański, 3 km na pn.-wsch. od Starogardu Gdańskiego, około 100 m n.p.m. Z przesłanych 185 dało się pomierzyć tylko 30 okazów, reszta była bez płatków lub zeschła i żółkła. 10.IX.1954.

Stanowisko 3. Kuwasy, pow. Grajewo, woj. białostockie, około 100–150 m n.p.m. Torfowisko niskie. Zebrano około 150 okazów, w przesyłce doszło zdalnych do wykonania pomiarów 77 okazów. 6.IX.1953.

Stanowisko 4. Okolice Łodzi, około 200 m n.p.m. Z przesyłki 167 okazów dało się pomierzyć tylko 34 sztuki, reszta doszła z zupełnie opadłymi płatkami. Około 5.IX.1954.

Stanowisko 5. Okolice Bydgoszczy, około 200 m n.p.m. Pomierzono tylko 26 egzemplarzy, reszta była zniszczona. 1.IX.1954.

Stanowisko 6. Zbocza Kobylego Wierchu nad miejscowością Tatrzańską Kotlina (Jaskinie Bielskie), 850 m n.p.m. Bardzo rzadki las mieszany na stromym zboczu, obok ścieżki turystycznej. Warunki bytowania roślin dobre, roślinność na ogół bujna, bogata w gatunki, biotop o charakterze naturalnym. Na wapieniu. 13.VIII.1953.

Stanowisko 7. Bukowina Tatrzańska, 860 m n.p.m. Mokra łąka nad Potokiem Odewsiańskim. Warunki niezbyt dobre, łąka corocznie koszona, bardzo podmoka. Na fliszu. 10.VII.1952.

Stanowisko 8. U stóp pn. zboczy Nosala, ponad Bystrem, 960 m n.p.m. Zacięzione polanki wśród młodego, rzadkiego lasu. Warunki dobre, biotop mniej więcej naturalny. Flisz. 4.VIII.1952.

Stanowisko 9. Wylot Stanikowego Żlebu, nieco powyżej Drogi pod Regłami, 1000 m n.p.m. Mała dolinka na skraju lasu nad brzegiem potoku. Warunki bytowania roślin dobre. Wapień. 25.VIII.1952.

Stanowisko 10. Przy szosie z Lysej Polany do Jaworzyny, około 1000 m n.p.m. Brzeg lasu, na przykopie, między świerkami. Ekspozycja pn. Warunki dość dobre. Duże zacięnienie i wilgotność. 11.IX.1954.

Stanowisko 11. Na pn. zboczu Krokwi, 1050 m n.p.m. Odsłonięte małe łączki przy leśnej drodze w lesie mieszanym o przewadze świerków. Warunki dobre. Dorywcze sporadyczne spasanie przez krowy. Wapień 2.IX.1952.

Stanowisko 12. W Dolinie Jaworowej, zrąb powyżej polany Gałajdówki, około 1080 m n.p.m. Warunki bardzo dobre, teren słoneczny, w miarę wilgotny, lekko zacięniony. Doskonała tłusta gleba. Na morenie granitowej. 15.VIII.1954.

Stanowisko 13. W Dolinie Białej Wody, przy drodze koło mostu na potoku Biała Woda, obok ujścia Doliny Spis-Michałowej, około 1090 m n.p.m. Wśród *Petasitetum*. Warunki dobre. Bujna łączka nad potokiem. Na morenie granitowej, tuż obok skałki jurajskiego wapienia, na kontakcie wapienia z granitem. Ekspozycja pd. i pd.-zach. 31.VIII.1954.

Stanowisko 14. Północne zbocze nad Przysłopem Kominiarskim w Dolinie Lejowej, 1150 m n.p.m. Wśród rzadkiego młodnika leśnego między trawą i siewkami świerków. Warunki dobre, biotop naturalny. Wapień. 10.VIII.1952.

Stanowisko 15. W Dolinie Białej Wody, obok ujścia Litworowego Żlebu, około 1190—1200 m n.p.m. Warunki bardzo dobre. Na granitowej morenie tłusta ilasta gleba, dostatecznie wilgotna, teren w miarę słoneczny. Przy ścieżce na wale morenowym obok potoku. 26.VIII.1954.

Stanowisko 16. Zimna Studnia w Dolinie Kiezmarskiej, 1230 m n.p.m. Duża polana, przy potoczku, na morenie granitowej. Warunki dobre, roślinność bujna. Na całej wielkiej polanie tylko jeden nieduży płat z dziewięciornikiem. 17.VIII.1954.

Stanowisko 17. Na Jaworzynce Miętusiej nad Doliną Miętusią, 1275 m n.p.m. Trawiaste upłazki na grani między małymi świerczkami. Warunki dobre, duże nasłonecznienie przy dobrej wilgotności i zacięniających krzewach; bujna roślinność nawapienna. 14.VIII.1952.

Stanowisko 18. Północne zbocze między Stołami a Przysłopem Kominiarskim, 1300 m n.p.m. Las świerkowy niezbyt gęsty. Warunki glebowe i mikroklimatyczne dobre, ale w lesie wypas krów i owiec. Na wapieniu. 10.VIII.1952.

Stanowisko 19. Hala Stoły nad Dolinką Kościeliską, 1340 m n.p.m. Polana wypasowa o ekspozycji wschodniej. Warunki średnie. Kwiaty zebrane z miejsca na uboczu, wśród drzew i krzaków, gdzie owce mniej chodzą. Na wapieniu. 10.VIII.1952.

Stanowisko 20. Koperszady Przednie pod Jatkami, około 1470 m n.p.m. Polana wśród kęp kosówki. Warunki na ogół dobre, ale miejsce wypasane w bieżącym roku. Upřednio nie było wypasu. Na wapieniu i kwarcycie. 16.VII.1954.

Stanowisko 21. Gładkie Uplaziańskie nad skałą Piecem, 1480 m n.p.m. Trawiasty, bardzo zerodowany i spasiony grzebiec z rzadkimi kępami kosówki. Bardzo kiepskie warunki, zupełnie zmieniony biotop, silna erozja całego terenu, całe połacie odsłoniętej kamienistej gleby — nadmierny wypas. Jedno z dwóch najuboższych zbadanych stanowisk. Na wapieniu. 24.VIII.1952.

Stanowisko 22. Jatki Przednie, około 1540 m n.p.m. Trawiaste zbocze z kępami kosówki, przy ścieżce. Warunki dobre. Na wapieniu. 16.VIII.1954.

Stanowisko 23. Gładkie Uplaziańskie między Piecem a Chudą Turnią, 1560 m n.p.m. Trawiaste, rozmyte i spalone zbocze z odsłoniętymi płatami piargu, w pobliżu małego źródelka. Bardzo kiepskie warunki bytowania, zupełnie zmieniony biotop, silna erozja terenu, całe połacie odsłoniętej kamienisto-żwirowatej gleby — nadmierny wypas owiec. Jedno z dwóch najuboższych zbadanych stanowisk. Na wapieniu. 24.VIII.1952.

Stanowisko 24. Dolina Białych Stawów, pd. zbocze Kopy Bielskiej, 1645 m n.p.m. Trawiaste polanki między kosówką, w pobliżu ścieżki. Miejsce, które jeszcze poprzedniego roku było wypasane, w bieżącym już nie. Warunki dobre. Na wapieniu. 12.VIII.1953.

Stanowisko 25. Przy ścieżce na Przełęcz pod Kopą od Doliny Białych Stawów, 1680 m n.p.m. Piarżysto-trawiaste zbocze o ekspozycji pd.-zach. Warunki bardzo dobre, teren dobrze nawilgocony a słoneczny, dobry odpływ, gruba warstwa dobrej gleby, bujna roślinność. Na kontakcie wapienia z granitem. 11.VIII.1953.

Stanowisko 26. Wielicka Dolina pod Mokłą Wantą (Granatnicą), od 1720 do 1750 m n.p.m. W trawie i ziołoroślach, na wilgotnym, ciągle zraszonym wodą podłożu granitowym. Warunki dobre. 19—20.VIII.1954.

Stanowisko 27. Pod wierzchołkiem Kominiarskiego Wierchu (Kominów Tylkowych), 1810 m n.p.m. Trawiaste upłazy wypasane. Warunki średnie, roślinność dość uboga i jednostajna, zwłaszcza w okolicy wierzchołka. Na piaszkowcach. 31.VIII.1952.

Stanowisko 28. Kominiarski Wierch, na wierzchołku, 1829 m n.p.m. Trawiaste upłazy wypasane. Warunki średnie, roślinność niezbyt bujna i dość jednostajna, spasana. Na piaszkowcach i wapieniu. 31.VIII.1952.

Stanowisko 29. Południowe, stromo nachylone zbocze Ciemniaka, na zejściu do Doliny Tomanowej, 1850 m n.p.m. Szeroka, długa depresja w rodzaju dość płytkiego, piarżysto-trawiastego żlebu. Warunki dość dobre. Na wapieniu. 24.VIII.1952.

Stanowisko 30. Wschodnie zbocze Kopy Kondrackiej pod grzbietem szczytowym, nad Doliną Kondratową, 1920 m n.p.m. Trawiaste upłazy. Teren spaszany, na kontakcie wapienia z granitem. Stanowisko *Parnassia* jeszcze na wapieniu, ale możliwe już wpływy chemiczne sąsiedztwa kwaśnej gleby granitowej. 25.VIII.1952.

Stanowisko 31. Poniżej wierzchołka Ciemniaka, od pd.-zach., 2030 m n.p.m. Zaczątek płaskiego, wilgotnawego, trawiastego żlebu. Warunki dość dobre, teren miejscami spaszany, zbocze strome, lawiniaste. Na wapieniu. 24.VIII.1952.

Stanowisko 32. Szeroka, płaska przełęcz między Małolączniakiem i Krzesanicą, 2040 m n.p.m. Trawiaste upłazki, skałki i płaty piargu. Na wapieniu. Warunki dość dobre, teren trochę spaszany, ale sporo miejsc nie zniszczonych wśród głazów i między skałkami. 25.VIII.1952.

Stanowisko 33. Wierzchołek Ciemniaka, 2096 m n.p.m. Trawiaste upłazki, przetykane kamieniami i żwirkiem. Warunki dość dobre, teren spaszany, ale pozostały nie uszkodzone miejsca między skałkami i kamieniami. Na wapieniu. 24.VIII.1952.

Prócz podanych wyżej, ostatnie, dodatkowe stanowisko z Puszczy Białowieskiej, na wysokości około 100 (?) m n.p.m., na nadrzecznej, mokrej łące wśród olch i zarosli wierzbowych, nie było terenem zbierania materiału. Dla pobieżnego porównania zmierzyłam tylko średnice kilku (około 10) kwiatów. Wszystkie te średnice przewyższały 30 mm.

Rozważając różnorodne czynniki, które mogą wpływać na rozwój kwiatów *Parnassia palustris*, a co za tym idzie, na wymiary ich koron, należy przede wszystkim podkreślić to, że kwiaty — celowo zbierane bez wyboru, w przypadkowej kolejności — znajdowały się w różnych stadiach rozwoju. Toteż niejednokrotnie, być może, pomiary chwyciły niecałkowicie jeszcze rozwinięte i nie osiągające swej ostatecznej wielkości płatki. Niemniej zwracałam uwagę na to, żeby nie brać nawet na pół rozwiniętych pąków oraz — jak to już zaznaczałam — w jakikolwiek sposób uszkodzonych egzemplarzy: nadjedzonych, niedorozwiniętych czy zdegenerowanych. W materiale zebrany były jednak kwiaty młode, gdzie jeszcze żaden pręcik nie rozpoczął rozwoju, ale były też i starsze, takie gdzie poodpadały już wszystkie pylniki, a nawet i płatki przy pomiarach zaczynały opadać.

W przeciwieństwie do np. *Campanula alpina* czy *C. polymorpha*, u których przy rozwijaniu się korony pręciki są już najczęściej zupełnie rozwinięte, a nawet częściowo pylniki wypylone, *Parnassia palustris* rozwija z pąka kwiatowego najpierw płatki, potem zaś dopiero pręciki.

Dla stwierdzenia, czy płatki rozwijają się nadal, gdy już pręciki rozpoczęły swój rozwój, dokonałam pomiarów na sześciu kwiatach: trzech rosnących w terenie, a równocześnie na trzech zerwanych i trzymanyh w szklance z wodą w pokoju. Wszystkie sześć kwiatów było w stadium rozwiniętych już płatków korony i rozpoczynającego właśnie swój rozwój pierwszego pręcika.

Wszystkie korony mierzyłam codziennie w ciągu sześciu dni. U kwiatów trzymanyh w pokoju jeden nie wykazał żadnego przyrostu przez cały czas, utrzymując średnicę korony początkową, tj. 23 mm. Drugi, o początkowej średnicy 20 mm, nie wykazał przyrostu pierwszego i drugiego dnia po zerwaniu, trzeciego dnia natomiast wykazał różnicę plus 3 mm, a więc osiągnął ostateczny wymiar średnicy 23 mm, po czym więcej już nie przyrastał. Trzeci kwiat, o średnicy początkowej 14 mm, wykazał drugiego dnia od zerwania przyrost 2 mm, a więc mierzył ostatecznie 16 mm i więcej już nie przyrastał.

Kwiaty rosnące w terenie również zachowywały się rozmaicie. Dwa, o średnicach 24 i 26 mm, nie wykazały przyrostu przez cały ciąg 6 dni pomiarów, trzeci kwiat natomiast, o początkowej średnicy 23 mm, wykazał drugiego dnia średnicę 24 mm, a więc przyrost 1 mm; dalszych zmian już nie wykazał.

Z powyższych przykładów możemy wnioskować, że część pomierzonego materiału mogła jeszcze nie osiągnąć swej ostatecznej wielkości.

Czynnik podłoża niewątpliwie ma również wpływ na rozmiar kwiatów. Większa część zebranego materiału pochodziła z gleb o odczynie alkalicznym, gdzie jednak poszczególne stanowiska różniły się z pewnością

Tabela 1 —

Średnica koron kwiatowych u *Parnassia palustris* L. w związku
Diameter of the flower corolla of *Parnassia palustris* L. in relation to the

Nr No.	Wysokość n.p.m. Height above sea-level	Liczba okazów Number of specimens	Kla Clas					
			10	13	16	19	22	25
	ca 100?	10						
1	10—20	60			2	6	11	24
2	ca 100	30				2	9	8
3	100—150	77			5	8	23	27
4	ca 200	34				10	14	5
5	ca 200	26				1	5	14
6	850	66				1	5	24
7	860	106				1	22	57
8	960	231				1	42	107
9	1000	61				1	19	24
10	1000	77			1	7	20	34
11	1050	131			1	25	56	45
12	1080	72					8	30
13	1090	100				3	20	50
14	1150	123			1	11	34	63
15	1190—1200	47					4	24
16	1230	60				6	17	20
17	1275	71					13	39
18	1300	73		1		6	33	27
19	1340	50			2	7	16	18
20	1470	28			1	2	11	12
21	1480	44			4	18	20	2
22	1540	81				4	31	33
23	1560	69		2	2	32	25	8
24	1645	92				3	32	41
25	1680	78					9	44
26	1720—1750	69		1		4	30	32
27	1810	55			1	17	26	11
28	1829	85			5	36	34	10
29	1850	138			2	27	72	34
30	1920	122			2	23	58	37
31	2030	92		1	7	51	33	
32	2040	165			9	48	77	29
33	2096	81	1		9	25	31	13

stopniem alkaliczności. Były to różne typy wapienia: muszlowy, krynoidowy, rogowcowy; były też dolomity, łupki, piaskowce itp., w sumie więc duża różnorodność materiału skalnego osadowego.

Gleby kwaśne, granitowe, z których zbierałam kwiaty dziewięciornika, były także różne: składał się na nie bądź typowy granit tatrzański, bądź zwietrzałe i rozkładające się łupki krystaliczne (gdzie nawet występowały

Table 1

ze wzniesieniem nad poziom morza na niżu i w Tatrach
height above the sea-level in the lowlands and in the Tatra Mountains

sy ses			Mo	Min — Max	M ± m	± σ
28	31	34				
					> 30	
11	5	1	25	15 — 34	24,76 ± 0,48	± 3,69
10	1		28	20 — 30	24,91 ± 0,55	± 3,03
14			25	15 — 29	23,44 ± 0,38	± 3,30
5			22	18 — 28	22,45 ± 0,51	± 3,00
4	2		25	20 — 30	25,12 ± 0,52	± 2,67
23	10	3	25	20 — 34	27,04 ± 0,38	± 3,06
24	2		25	20 — 30	25,12 ± 0,21	± 2,19
73	8		25	20 — 32	25,57 ± 0,15	± 2,37
16	1		25	20 — 32	24,85 ± 0,32	± 2,52
14	1		25	17 — 30	24,19 ± 0,32	± 2,82
4			22	17 — 28	22,60 ± 0,21	± 2,40
16	16	2	25	22 — 33	26,92 ± 0,36	± 3,09
22	5		25	20 — 31	25,18 ± 0,26	± 2,58
11	3		25	17 — 30	23,74 ± 0,26	± 2,91
16	2	1	25	21 — 34	26,20 ± 0,35	± 2,37
12	5		25	20 — 31	24,64 ± 0,43	± 3,30
16	2	1	25	21 — 33	25,42 ± 0,28	± 2,37
6			22	12 — 28	23,23 ± 0,30	± 2,58
5	2		25	16 — 30	23,38 ± 0,47	± 3,30
2			25	16 — 27	23,29 ± 0,49	± 2,58
			22	15 — 25	20,35 ± 0,33	± 2,19
11	2		25	20 — 30	24,10 ± 0,29	± 2,58
			19	13 — 26	20,53 ± 0,30	± 2,52
14	1	1	25	20 — 35	24,37 ± 0,27	± 2,61
18	7		25	21 — 31	25,87 ± 0,27	± 2,37
2			25	14 — 28	23,26 ± 0,23	± 2,31
			22	17 — 26	21,55 ± 0,31	± 2,28
			19	15 — 25	20,74 ± 0,25	± 2,28
3			22	16 — 27	22,21 ± 0,19	± 2,25
2			22	17 — 27	22,33 ± 0,21	± 2,37
			19	14 — 23	19,78 ± 0,20	± 1,92
2			22	15 — 28	21,40 ± 0,19	± 2,49
2			22	11 — 27	20,89 ± 0,34	± 3,09

niektóre rośliny, tzw. wapienne), niekiedy pojawiała się domieszka bardzo kwaśnego pegmatytu itp.

Wilgotność podłoża stanowiła też czynnik zmienny. Większa przy źródłiskach, nad potokami, w wysokiej, bujnej trawie wśród młodników leśnych, w cieniu skałek — znacznie zaś mniejsza, często minimalna, na odsłoniętych zboczach, wśród kamienistych upłazków, na ledwie poro-

śniętych żwirkach, a zwłaszcza na podłożu silnie zerodowanym i spasionym.

Ekspozycja zboczy oczywiście także odgrywała pewną rolę. Zbocza północne, chłodniejsze i ciemniejsze, o dłużej zalegających płatach śnieżnych, stwarzają inne warunki dla kwitnienia roślin niż zbocza południowe, nasłonecznione. Może to mieć bardzo wydatny wpływ na wielkość kwiatów.

Wreszcie niezmiernie ważnym czynnikiem jest spasanie przez owce. W terenie wypasany, nawet bez szczegółowszych badań, rzuca się od razu w oczy zubożenie roślinności. W miejscach, gdzie odbywa się stały wypas owiec czy też krów, można było zaobserwować, zanim jeszcze dokonano ścisłych pomiarów, że rośliny są niższe, a kwiaty ich mniejsze. Tak np. stanowiska 21 i 22 na Gładkiem Uplaziańskim były ze wszystkich zbadanych bodaj najbardziej zniszczone i najuboższe: rośliny niewyrośnięte, ogryzione, mało gatunków, kwiaty małe i nieliczne. Bardzo jaskrawe obniżenie tam wielkości kwiatów *Parnassia* należy najprawdopodobniej przypisać owemu zubożeniu, wywołanemu przez wyjątkowo nadmierny wypas.

Te wszystkie wpływy, których szczegółowsze zbadanie wychodzi poza ramy niniejszej pracy, powinny być w badaniach moich do pewnego stopnia przynajmniej zostać o tyle o ile zrównoważone przez dobieranie rozmaitych stanowisk o tej samej lub mało się różniącej wysokości, co też w miarę możliwości, czyniłam.

Najdoskonalszym rozwiązaniem tego problemu byłaby możliwość mierzenia koron kwiatowych z roślin wzdłuż jednego ciągu na jednym i tym samym zboczu o możliwie jednakowych warunkach siedliskowych. Jest to jednak, oczywiście, niemożliwe, tak z racji braku podobnie długiego i rozległego, a jednolitego pod każdym względem zbocza, jak i z powodu występowania dziewięciornika w terenie tylko na stosunkowo niewielkich płatach.

Na stanowiskach niżowych, skąd uzyskałam materiał do niniejszej pracy, uderza wyjątkowa małość koron kwiatowych. Jeśli chodzi o stanowisko 3, z Kuwasów, to według P a w ł o w s k i e g o (ustne dane), można to zjawisko przypisać wyjątkowo nędznemu, ubogiemu podłożu łąk pomelioracyjnych na torfowisku. Niezależnie jednak od tego, dla wszystkich przesłanych kwiatów czas i warunki ich przesyłki mają z pewnością również wpływ na wielkość mierzonych koron. Regułą w przesyłkach było to, że około dwóch trzecich roślin z każdej paczki ulegało zupełnemu zniszczeniu, płatki opadały lub marszczyły się i zsychały. Te kwiaty, które w chwili wysyłki były w pełni rozwoju, opadały i schły w okresie doręczenia, a tylko te, które przy wysyłaniu zaczynały się dopiero rozwijać, dochodziły w stanie jako tako zadowalającym. Sądzę, że jednak róż-

wój ich po zerwaniu i w warunkach przesyłki musiał zostać albo zahamowany, albo co najmniej niezupełny. Tym można by tłumaczyć te, tak małe średnice koron u przesyłanych roślin, podczas gdy na świeżo, w warunkach naturalnych, np. w Puszczy Białowieskiej, kwiaty *Parnassia* uderzały wielkością koron, bujnym wyglądem, wysokością łodyg. Obserwacje te jednak czyniłam wówczas w Puszczy dorywczo, przed podjęciem niniejszej pracy.

Ujęcie cytologiczne wielkości kwiatów *Parnassia palustris* dał w swej pracy Erlandsson (1942). Przebadał on dziewięciorniki z terenu Skandynawii i Danii i stwierdził, że występują tam trzy formy różniące się między sobą cytologicznie i pokrojowo: *Parnassia palustris* forma typica, która może być 1) diploidem ($2n = 18$) lub 2) tetraploidem ($2n = 36$) oraz skandynawska odmiana 3) *P. palustris* var. *tenuis*, która jest tetraploidem. Obserwacje morfologiczne wykazały widoczne i znaczne różnice między diploidami i tetraploidami *P. palustris*; tetraploidy mianowicie mają znacznie większe liście i kwiaty. *P. palustris* var. *tenuis* (która jest tetraploidem) jest trochę mniejsza od diploidu formy typowej, ale płatki i liście ma nieco od niego szersze.

Wszystkie te trzy formy różnią się też rozmieszczeniem: diploidy formy typowej występują głównie na południu kraju, tetraploidy na północy, zaś var. *tenuis* w Górach Skandynawskich, na Wybrzeżu Arktycznym i na brzegach Zatoki Botnickiej.

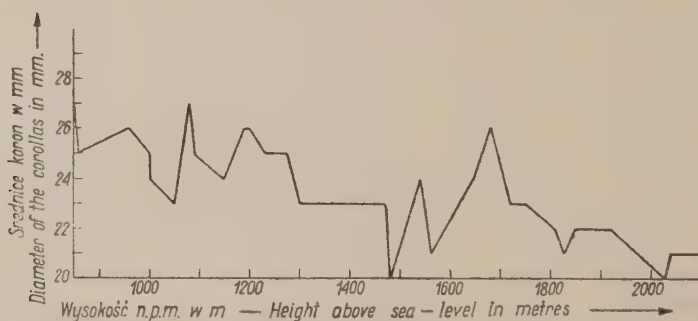
Na podstawie swych badań Erlandsson uważa, iż powinno się dzielić gatunek *Parnassia palustris* na trzy rasy odpowiadające typom chromosomowym, i powołuje się przy tym na zdanie Nannfeldta: „ponieważ rasy chromosomowe wyróżniają się morfologicznie i są rozpoznawalne dla systematyka, powinny być raczej uważane za gatunki, nawet gdy cechy morfologiczne są nieznaczne“.

W celu poznania kariologii *Parnassia palustris* w Polsce przeprowadziła specjalne badania E. Banach-Poganowa (1956). Miło mi tu złożyć serdeczne podziękowanie zarówno prof. dr M. Skalińskiej za zainicjowanie tych badań i za udzielone mi cenne uwagi, jak i dr E. Pogonowej za użyczenie wyników pracy, jeszcze przed jej publikacją.

Opracowany przez Pogonową materiał obejmuje *Parnassia* zebraną z 15 naturalnych stanowisk, w tym 7 stanowisk z Tatr wapiennych i granitowych, do wysokości 1750 m. Wszystkie przebadane rośliny okazały się diploidami o liczbie chromosomów $2n = 18$. Pogonowa stwierdza, że występowanie diploidalnego typu *Parnassia palustris* L. na obszarze Polski jest zgodne z ogólnym rozmieszczeniem kariotypów tego gatunku.

Zatem czynnik kariologiczny nie odgrywa żadnej roli w wielkości kwiatów *Parnassia palustris* w Tatrach i na terenie całej Polski.

Zestawienie i przeliczenie w tabeli 1 dokonanych pomiarów średnic koron kwiatowych oraz uwidoczniona na ryc. 1 krzywa zmian średnic tychże koron wykazują dość wyraźnie, że kwiaty dziewięciornika, na ogół, zmniejszają się rzeczywiście w miarę wzrastania wysokości stanowisk tej rośliny nad poziom morza. Zmniejszanie to nie występuje jednak proporcjonalnie do wysokości, ponieważ prócz czynnika wysokości w grę wchodzi inne jeszcze czynniki siedliskowe, które wpływają bądź dodatnio, bądź ujemnie na rozwój i wielkość kwiatów i całej rośliny. Wpływ tych dodatkowych czynników może powodować wahania in plus lub in minus



Ryc. 1. Krzywa zmian średnicy koron kwiatowych *Parnassia palustris* L. w Tatrach
Curve illustrating the changes in the diameter of the flower corolla of *Parnassia palustris* L. in the Tatra Mountains

w kolejnych — według wysokości n.p.m. — liczbach obrazujących przeciętną średnicę kwiatu na tej wysokości. Liczby te wykazują niekiedy uderzające odskoki od przeciętnej.

Podkreślić należy, że dobrze uchwytnym, a silnie negatywnym czynnikiem, wpływającym na rozwój roślin i wielkość kwiatów, jest nadmierny wypas owiec na obszarach, gdzie występuje *Parnassia palustris*.

PRĄTNICZKI (STAMINODIA)

Zarówno na świeżych, jak i na zasuszonych kwiatach dziewięciornika zauważyłam, że ilość frędzlastych, nitkowatych rozgałęzień prątniczek jest zmienna. Przy tym nie tylko poszczególne prątniczki jednego i tego samego kwiatu różnią się między sobą ilością owych rozgałęzień, ale także suma tych rozgałęzień u jednych kwiatów jest większa, u innych zaś mniejsza. Chciałam więc zbadać, czy nie zachodzi korelacja pomiędzy tą ilością a wzniesieniem n.p.m. stanowisk kwiatów.

Na części zasuszonych kwiatów mogłam z dość dużą łatwością prze-liczyć pod lupą, a nawet gołym okiem, wszystkie rozgałęzienia prątnicz-

ków, część okazów zaś rozmoczyłam w ciepłej wodzie, po czym wyparowałam oddzielnie każde staminodium dla dokonania obliczeń.

W języku polskim nie ma odpowiednich nazw dla poszczególnych części prątniczków u *Parnassia palustris*, nie ma ich zresztą w ustalonej formie i w innych językach. Kuliste zakończenia rozgałęzień nazywa się czasem gruczołkami — niesłusznie, bo niczego nie wydzielają; czasem pęcherzykami, także niezbyt szczęśliwie, bo — jak wykazały szczegółowe badania Daumanna (1936) — nie mają one wewnątrz wolnej przestrzeni, tylko tkankę mięksizową o charakterze częściowo palisadowym, częściowo gąbczastym.

Będę więc używała nazwy „kulki“ dla kulistych zakończeń na rozgałęzieniach prątniczków, nazwy zaś „nitki“ dla wydłużonych części tych rozgałęzień, dźwigających na końcach owe kulki. Utwory te zresztą ontogenetycznie odpowiadają rzeczywiście nitkom przecików, przynajmniej w odniesieniu do środkowego z tych utworów (Daumann l.c.). Każde rozgałęzienie prątniczka, tj. nitkę łącznie z kulką nazywać będę „palieczką“.

Dolna, nasadowa część prątniczka prawie się nie różni od nasady zwykłego przecika u *Parnassia*, jest tylko może nieco węższa. W odległości 1—2 mm od nasady rozszerza się z nagle kolistawo, tworząc powierzchnię właściwego miodnika, wydzielającą nektar, zresztą w niewielkich ilościach. Miodnik ów przedstawia jak gdyby dwie mniej lub więcej symetryczne połówki, czasem bardziej, czasem mniej wyraźnie rozgraniczone. Obie powierzchnie miodnika: dolna od strony płatków i górna od strony słupka, są lekko wypukłe (dolna zwykle nieco silniej), ze słabo zaznaczoną wklęsłością wzdłuż środkowej, pionowej osi, po obu jej stronach. Miodnik ma intensywnie szmaragdowozieloną barwę; na górnym brzegu wystrzępiony jest w rodzaj długiej, delikatnej frędzli, utworzonej z szeregu cienkich białawych nitek, z których każda jest zakończona mniej więcej kulistym utworem — kulką. Według moich obserwacji kulki te na nitkach środkowych prątniczka są zwykle drobniejsze i bardziej wydłużone przypominając niekiedy kształt żółdzi lub małych buteleczek. Na bocznych nitkach kulki są zwykle największe, bardziej spłaszczone, w kształcie zbliżone do krótkiej, grubej gruszcзки. Dolne zaś, najmniejsze, są już wyraźnie kuliste. Na samym brzegu po obu stronach miodnika często zdarzają się po jednej lub po dwie małe, czasem prawie siedzące, bez nitek, zupełnie przezroczyste kuleczki (ryc. 2). Z wyjątkiem tych ostatnich, każda kulka — jak to opisał Daumann (l.c.) — zawiera w środkowych komórkach swej dolnej części liczne żółtozielone chromatofofy. Otaczająca je warstwa komórek palisadowych silnie łamie światło powodując połyskliwość kulek.

Nitka środkowa przedłuża się na powierzchnię miodnika uwypuklając się na niej czasem silniej, czasem słabiej i dzieląc ją w ten sposób na wspomniane wyżej dwie połówki.

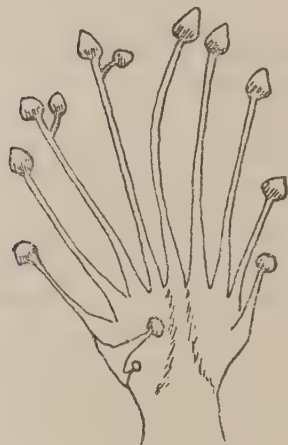
Długości nitek staminodialnych są różne: na kwiatach ze stanowisk niższych — jak to zaobserwowałam — zawsze dłuższe, kulki zaś wtedy są bardziej wydłużone, buteleczkowate; na wysokich stanowiskach nitki są krótsze, a kulki okrągłejsze; cały prątniczek jest bardziej zwarty, „krępy“.

Wzajemna długość nitek w jednym prątniczku także się różni: czasem wszystkie nitki są niemal równe, a czasem boczne są znacznie krótsze w porównaniu z długimi środkowymi.

Wcięcia nitek w brzeg miodnika bywają czasem głębsze w samym jego środku i takie głębsze wcięcia podkreśla i uplastycznia połówki miodnikowe; kiedy indziej jednak wszystkie wcięcia są mniej więcej jednakowe.

W kwiatach młodszych, gdzie dopiero zaczynają się odginać pręciki, kulki są bardziej zielone, w starszych kwiatach natomiast jaśnieją, żółkną; wyjątkowo różnice te nie występują.

W morfologicznej budowie prątniczek zdarzają się czasem pewne odstępstwa od normy. Np. w jednym prątniczku dwie z nitek rozgałęziały



Ryc. 2. Pojedynczy prątniczek. Widać rozdwójenia środkowych nitek oraz małąk przezroczystą kulkę z lewej u dołu

Single staminode. Noticeable are bifurcations of two filaments, and a small transparent "globule" (lower left)

się tuż pod szczytem tworząc każda po dwa końce, opatrzone kulkami normalnej wielkości (ryc. 2). W niektórych znów prątniczkach zdarzały się dodatkowe nitki, blisko o połowę krótsze od pozostałych, zwykłych, wyrastające przed nimi, niejako w drugim rzędzie i posiadające nieco mniejsze od normalnych kulki (ryc. 3).

Dla porównania ilości pałeczek w prątniczkach u kwiatów z różnych wysokości n.p.m. policzyłam owe pałeczki w dwóch seriach kwiatów, w każdej po 6 sztuk; w pierwszej ze stanowiska na wysokości 1000 m n.p.m. (9 stanowisko, wylot Stanikowego Żlebu), w drugiej zaś z wyso-

kości 1850 m n.p.m. (29 stanowisko, zbocze Ciemniaka). Zestawienie obliczeń podaje w załączonej tabeli 2.

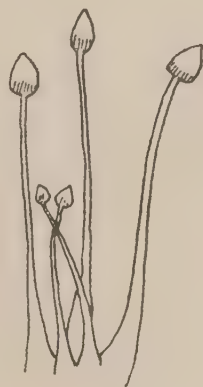
Z tabeli tej wynika, że w badanym materiale z wysokości 1000 m n.p.m. wyraźnie przeważa większa liczba pałeczek w każdym poszczególnym prątniczku i dochodzi niekiedy do 18 sztuk w jednym. Na wysokości 1850 m n.p.m. natomiast w prątniczkach badanych ilość pałeczek jest znacznie mniejsza, dochodząca najwyżej do 12 pałeczek w jednym prątniczku.

Sprawdzałam jeszcze te zmiany ilości pałeczek na licznych egzemplarzach zielnikowych kwiatów z różnych wysokości n.p.m. — i wszystkie kwiaty z wyższych stanowisk wykazywały mniejsze ilości pałeczek w prątniczkach niż egzemplarze ze stanowisk niższych. Na stu kilkudziesięciu egzemplarzach stwierdziłam stałe powtarzanie się tego zjawiska.

Tak więc na podstawie obserwacji dokonanych na stu kilkudziesięciu kwiatkach ze stanowisk na różnych wysokościach, jak też na podstawie zestawienia porównawczego (tabela 2) można przyjąć, że wraz ze wzrostem wysokości n.p.m.: a) zmniejsza się ilość pałeczek w każdym prątniczku,

Ryc. 3. Dwie dodatkowe nitki z kulkami stanowiące drugi rząd

Two additional filaments with globules (rodlets) forming a second row



niczku, b) kulki bardziej się zaokrąglają, nie są tak buteleczkowate jak na stanowiskach niższych, c) nitki stają się coraz krótsze.

Ilość pałeczek każdego prątniczka w jednym kwiecie jest zmienna, przy czym różnice zaobserwowane przeze mnie wahają się w granicach od 1 do 6 pałeczek.

Erlandsson (l.c.) podaje zestawienie pałeczek w prątniczkach u *Parnassia palustris* f. *typica*, według różnych autorów. Hartman (1879) podaje ich 10 do 15, Lange (1886—1888) — 9 do 13, Neuman (1901) — 9 do 25, Hiitonen (1933) — 12 do 25, Komarow (1939) — 9 do 13, Nordhagen (1940) — 9 do 25. Niestety, autor zestawienia nie podał szczegółów ani wysokości, skąd pochodziły mierzone kwiaty.

Tabela 2 — Table 2

Zestawienie ilości pałeczek w prątniczkach *Parnassia palustris* L. w związku z wysokością stanowisk nad poziom morza
Comparison of the number of rodlets in the staminodes of *Parnassia palustris* L. in relation to the height above sea-level

Wysokość n.p.m. w metrach Height above sea-level in metres	Liczba pąteczek w prątniczkach poszczególnych kwiatów Number of rodlets in the staminodes of separate flowers																				Przeciętna liczba pąteczek w prątniczku Mean number of rodlets in the staminode											
	kwiat flower I					kwiat flower II					kwiat flower III					kwiat flower IV					kwiat flower V					kwiat flower VI					13,5	
	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e	a	b	c	d	e		
1000	8	11	11	11	14	13	15	15	15	18	10	13	13	13	13	14	15	15	17	18	12	14	14	14	14	15	11	12	13	14	14	10,8
1850	9	10	11	12	12	11	12	12	12	12	11	11	11	11	11	12	9	10	10	11	11	9	11	11	11	11	9	9	10	11	11	

nie wiadomo więc, czy różnice te dotyczą poszczególnych kwiatów czy różnych wysokości.

Częstym zjawiskiem jest zależność barwy kulek od dojrzałości kwiatu: w kwiatach młodszych kulki są intensywniej zielone, u starszych — zwykle jaśniejsze, żółtozielone lub żółtawe.

STRESZCZENIE

Z badań i obserwacji w niniejszej pracy wynika, że u *Parnassia palustris* L.:

- 1) W miarę wzrostu wysokości stanowisk nad poziom morza:
 - a) zmniejsza się średnica koron kwiatowych, przy czym — według badań E. Banach-Pogan — czynnik kariologiczny nie odgrywa w tym roli,
 - b) zmniejsza się ilość pałeczek w każdym prątniczku,
 - c) skraca się długość nitek w prątniczkach,
 - d) kulki w prątniczkach zaokrąglają się, w przeciwstawieniu do butelczkowato wydłużonych kulek ze stanowisk niskich.
- 2) Ilość kulek w poszczególnych prątniczkach jednego kwiatu jest zmienna.
- 3) Kwiaty odznaczają się prawidłową formą, anormalności zdarzają się rzadko.
- 4) W miarę rozwoju i dojrzewania kwiatu zmienia się (jaśnieje) barwa kulek w prątniczkach.
- 5) Wbrew dotychczasowemu pogładowi stwierdzono, że:
 - a) *Parnassia* występuje po południowej stronie Tatr Wysokich: w Dolinie Kiezmarskiej i Wielickiej,
 - b) na południowych stokach Tatr Bielskich *Parnassia* występuje bardzo obficie.

Na koniec stwierdzono ponad wszelką wątpliwość, że wypas bydła i owiec na łąkach i halach tatrzańskich odgrywa wybitnie ujemną rolę w rozwoju *Parnassia palustris* (jak zresztą i innych roślin), a zwłaszcza wpływa hamująco na rozwój jej kwiatów i wielkość koron kwiatnych.

Panu prof. drowi Bogumiłowi Pawłowskiemu dziękuję najserdeczniej za cenne rady i uwagi, udzielane mi w czasie trwania pracy. Składam również podziękowanie pani prof. dr Janinie Szaferowej za rady i wskazówki dotyczące pomiarów biometrycznych.

Tatrzańska Stacja Naukowa
Zakład Ochrony Przyrody PAN

(Wpłynęło: 6.12.1959 r.)

SUMMARY

The author's studies, carried out on *Parnassia palustris* L. in the Tatra Mountains, brought the following results:

- 1) Parallel to the rise of height above sea-level of the stations from which the plants were collected, there was observed:
 - a) decrease of the diameter of the flower corolla (cf. Table 1, Fig. 1); (the karyological factor plays in it no role);
 - b) decrease of the number of rodlets (globule-bearing filaments) in each stamino-node (cf. Table 2);

- c) shortening of the length of the filaments in the staminodes;
- d) the globules in the staminodes become round, in contrast to the bottle-like elongated globules in flowers from low-lying localities.
- 2) The number of rodlets in the various staminodes of a single flower is not the same.
- 3) The flowers are mostly of normal shape, abnormalities being rare.
- 4) As the flower develops and becomes mature, a change takes place in the colour of the globules in the staminodes.
- 5) Contrary to data hitherto published, the author discovered that *Parnassia palustris* does grow on the southern slopes of the High Tatras (in the Wielicka Valley and in the Kieżmarska Valley), and that it occurs very abundantly on the southern slopes of the Bielskie Tatras.

Finally, it was ascertained beyond all doubt that cow- and sheep-grazing on the meadows and alps of the Tatra Mountains exerts a distinctly harmful effect upon the development of *Parnassia palustris*, especially checking the development of its flowers.

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Odrębność gatunkowa i próba wyjaśnienia genezy *Alisma lanceolatum* With.

The origin of Alisma lanceolatum With. in light of karyological
and morphological studies

E. POGAN

Rodzaj *Alisma* L. stanowi od wielu lat przedmiot żywego zainteresowania botaników, zarówno systematyków, jak i karyologów. Z przeglądu obszernej literatury, dotyczącej systematyki rodzaju *Alisma* L. (M. Micheli 1881, F. Buchenau 1903, G. Hegi 1906, P. Ascherson i P. Gräbner 1913, M. Raciborski i W. Szafer 1919, G. Samuelsson 1922, 1932, 1933, H. Glück 1936, M. L. Fernald 1950, W. Szafer, S. Kulczyński, B. Pawłowski 1924, 1953, A. J. Hendricks 1957, J. Dostal 1958 i inni) wynika, iż poszczególni autorzy wyrażają różne poglądy w odniesieniu do wartości systematycznej wyróżnianych o obrębie tego rodzaju taxa. Przyczyną tych rozbieżności jest, obok innych, trudność w wyborze odpowiednich kryteriów diagnostycznych. Niektóre z cech morfologicznych, uderzające na pierwszy rzut oka (np. wielkość roślin, wielkość liści, pewne cechy ich kształtu), nie mają w istocie wartości diagnostycznej. Cechy te bowiem wykazują u *Alisma* (podobnie jak u wielu roślin wodnych i błotnych) znaczną plastyczność, a określane na ich podstawie taxa okazały się tylko modyfikacjami.

Natomiast, jak to już podkreślał Samuelsson (1932), doskonałymi cechami diagnostycznymi dla gatunków rodzaju *Alisma* są drobne wprowadzie, ale bardzo stałe cechy dotyczące budowy kwiatów i owoców.

W oparciu o bogate materiały zielnikowe, pochodzące z całego świata, Samuelsson (1932) wyróżnił w obrębie rodzaju *Alisma* L. 6 mniej lub bardziej dobrych gatunków, 3 podgatunki i 1 odmianę:

Alisma plantago-aquatica L. (typicum)

A. plantago-aquatica L. ssp. *orientale* Sam. n. comb.

A. plantago-aquatica L. ssp. *brevipes* (Greene) Sam. n. comb.

Alisma lanceolatum Withering

Alisma canaliculatum A. Braun et Bouché

Alisma rariflorum Sam. n. sp.

Alisma subcordatum Rafinesque*Alisma gramineum* Gmelin (typicum)

A. gramineum Gmel. var. *Geyeri* (Torrey) Sam. n. comb.

A. gramineum Gmel. ssp. *Wahlenbergii* Holmberg.

Samuelsson zaznacza w odniesieniu do grupy 5 pierwszych gatunków, iż pokrewieństwo pomiędzy nimi jest bliższe niż pokrewieństwo jakiegokolwiek gatunku tej grupy z *A. gramineum*. *A. canaliculatum*, *A. rariflorum*, *A. subcordatum* i *A. gramineum* są ostro odgraniczone od

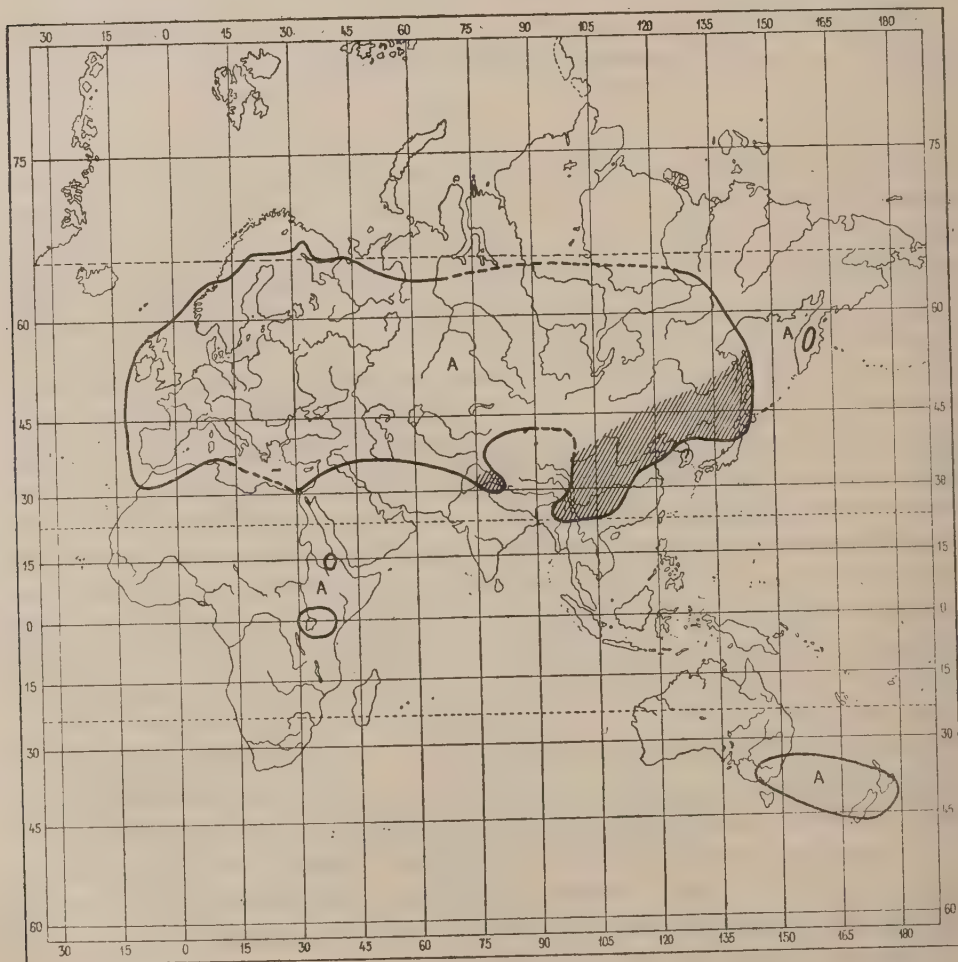


Fig. 1. Rozmieszczenie geograficzne *Alisma plantago-aquatica* L. A — *A. plantago-aquatica* L. (typicum); w obszarze zakreskowanym głównie ssp. *orientale* Sam.; — — — — granica nieokreślona (wg Samuelssona, 1933)

Area of distribution of *Alisma plantago-aquatica* L. A — *A. plantago-aquatica* L. (typicum); in the hatched part — mainly ssp. *orientale* Sam.; — — — — the limit undefined (after Samuelsson, 1933)

siebie i od pozostałych dwu gatunków. Natomiast mniej wyraźnie zaznacza się w cechach morfologicznych odrębność gatunkowa *A. lanceolatum* w odniesieniu do *A. plantago-aquatica*. Według Samuelssona różnice morfologiczne pomiędzy tymi dwoma gatunkami są tak nieznaczne, że

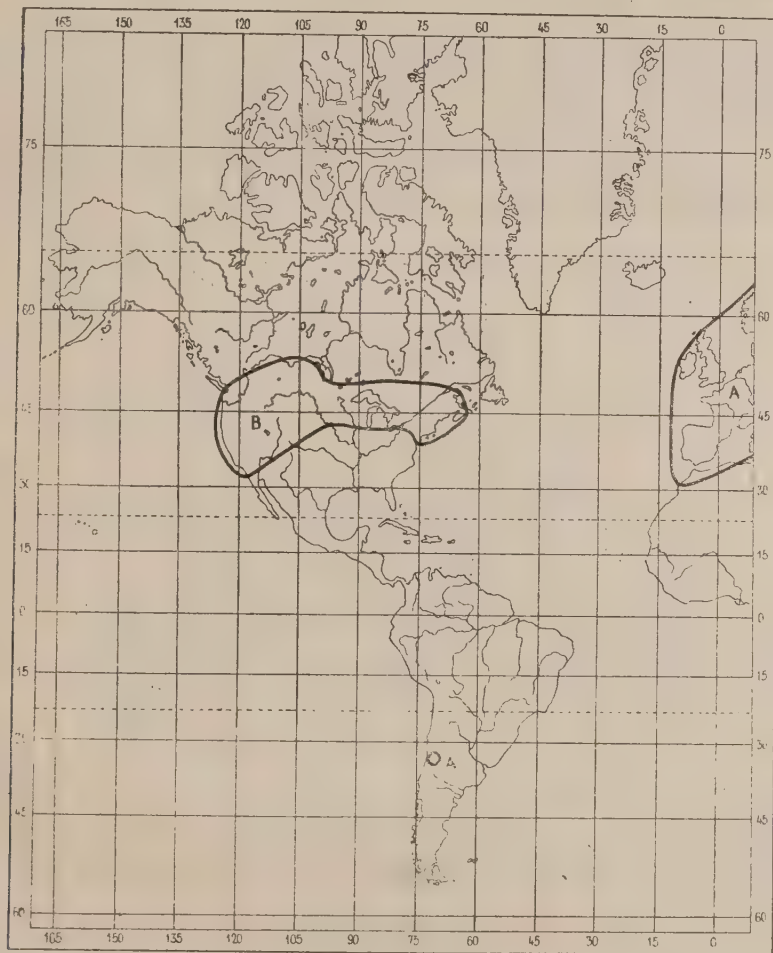


Fig. 2. Rozmieszczenie geograficzne *Alisma plantago-aquatica* L. A — *A. plantago-aquatica* L. (typicum); B — ssp. *brevipes* (Greene) Sam. (wg Samuelssona, 1933)

Area of distribution of *Alisma plantago-aquatica* L. A — *A. plantago-aquatica* L. (typicum); B. — ssp. *brevipes* (Greene) Sam. (after Samuelsson, 1933)

można by powątpiewać, czy *A. lanceolatum* zasługuje na rangę gatunku; większość autorów uważała ten taxon tylko za odmianę. Jednakże za odrębnością gatunkową *A. lanceolatum* przemawia bardzo wyraźnie charakter zasięgu tego gatunku.

A. plantago-aquatica, łącznie z obydwoma podgatunkami ma zasięg najszerszy (fig. 1, 2). Obejmuje on niemal całą Europę i prawie całą Azję, śródziemnomorską część Afryki Pn., i pewne, wyżej położone tereny na obszarach równikowej Afryki Wschodniej, zachodnią część Ameryki Pn., pewien obszar pd.-wsch. Kanady i pn.-wsch. stanów USA.

A. lanceolatum (fig. 3) występuje w śródziemnomorskiej części Europy, Afryki, w krajach bałkańskich, na obszarze środkowej Europy; na północy dochodzi do Anglii, Szwecji, Finlandii. Na wschodzie występuje w pd.-wsch. i środkowej Azji oraz w Małej Azji. W obszarze śródziemnomorskim *A. lanceolatum* jest gatunkiem znacznie częstszym niż *A. plantago-aquatica*. Występowanie *A. plantago-aquatica* (typicum) i *A. lanceolatum*

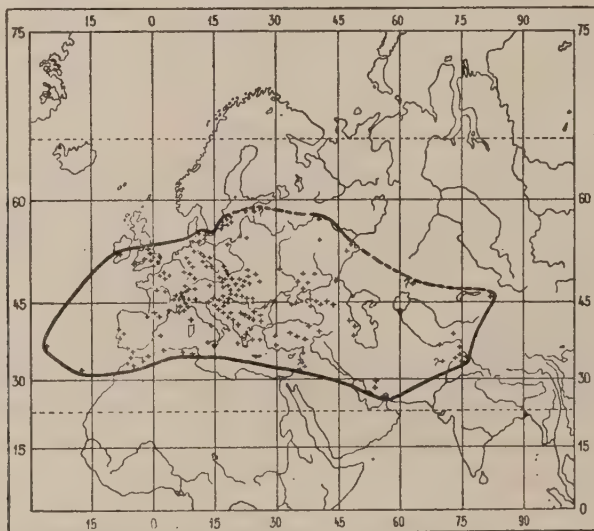


Fig. 3. Rozmieszczenie geograficzne *Alisma lanceolatum* Withering. (? — egzemplarze niepewne); — — — granica nieokreślona (wg Samuelssona, 1933)
 Area of distribution of *Alisma lanceolatum* Withering. (? uncertain specimens);
 — — — limit undefined (after Samuelsson, 1933)

w Ameryce Samuelsson tłumaczy ich zawleczeniem na te tereny przypuszczalnie w czasach nowszych. Prawdopodobnie również zawleczona została *A. plantago-aquatica* do Australii, wydaje się jednak że w czasach dawniejszych.

Bliskie pokrewieństwo systematyczne obu omawianych gatunków, jak również zasięg *A. lanceolatum* samodzielny, znacznie mniejszy i mieszczący się w obrębie zasięgu *A. plantago-aquatica* (typicum) wskazują na to, iż ten gatunek mógł brać udział w wytworzeniu gatunku *A. lanceolatum*.

Z badań kariologicznych wynika, że zarówno *A. plantago-aquatica*, jak i *A. lanceolatum* wykazują zróżnicowanie śródgatunkowe (tabela 1). Obok najczęściej podawanej dla *A. plantago-aquatica* liczby $2n = 14$, zna-

Tabela 1 — Table 1

Liczba chromosomów gatunków rodzaju *Alisma* L.Chromosome numbers of the species of the genus *Alisma* L.*Alisma plantago-aquatica* L.

Anglia	2n = 14	Heppel, nie publ. wg P. Maude, 1939
	2n = 14	A. A. Priestley, 1953
Szwecja	2n = 12	A. Löve i D. Löve, 1942
	2n = 12	O. Palmgren, 1943
	n = 6	
	2n = 12	I. Blomstrand, nie publ., wg A. Löve i D. Löve, 1944.
	2n = 14	
	2n = 14	S. Erlandsson, 1946
	2n = 14	A. Löve i D. Löve, 1948
	2n = 14	A. Löve, 1954
Finlandia	2n = 14	S. Erlandsson, 1946
Dania	n = 7	O. Hagerup, 1944
	2n = 14	S. Erlandsson, 1946
	2n = 14	E. Tschermak-Woess, 1948
Niemcy	2n = 10	H. D. Wulff, 1939
	n = 7, 8	
	2n = 14, 16	H. D. Wulff, 1950
Austria	2n = 14	E. Tschermak-Woess, 1948
	2n = 14	K. Holzer, 1952
Węgry	2n = 14	L. Pólya, 1949
Rumunia	2n = 12	J. T. Tarnavski, 1948, wg A. Löve i D. Löve, w druku
Portugalia	2n = 14	D. de Castro i N. M. Noronha-Wagner, 1950, wg A. Löve i D. Löve, w druku.
Ameryka Pn.		
Wisconsin	2n = 14	E. Oleson, 1941
Columbia Co.		

Materiały z Ogrodów Botanicznych oraz inne o nie zidentyfikowanym pochodzeniu

Niemcy, Kilonia	n = 14	H. D. Wulff, 1950
Ogr. Bot.	2n = 28	
Włochy, Piza	2n = 14	H. D. Wulff, 1950
Ogr. Bot.		
—	2n = 10	Wewetzer, 1956
—	2n = 12	O. Liehr, 1916
—	2n = 14	A. J. Hendricks, 1957

Alisma plantago-aquatica L. var. *americanum* J. A. Schultes et Schult

—	2n = 14	A. J. Hendricks, 1957
—	2n = 28	

(Tabela I. c. d.)

Alisma lanceolatum With.

Anglia	2n = 28	A. A. Priestley, 1953
Szwecja	2n = 28	S. Erlandsson, 1946
	2n = 28	A. Löve i D. Löve, 1948
Dania	n = 14	O. Hagerup, 1944
	2n = 28	S. Erlandsson, 1946
	2n = 26	E. Tschermak-Woess, 1948
	n = 13	
Austria	2n = 26	E. Tschermak-Woess, 1948
	n = 13	
Rumunia	2n = 24	Tarnavski 1948 (wg A. Löve i D. Löve w druku)
Portugalia	2n = 26	D. de Castro i N. M. Noronha-Wagner, 1950, wg A. Löve i D. Löve, w druku

Materiały z Ogrodów Botanicznych oraz inne o nie zidentyfikowanym pochodzeniu

Szwecja, Uppsala	2n = 26	A. Löve i D. Löve, 1944
Ogr. Bot.	2n = 28	S. Erlandsson, 1946

Alisma gramineum Gmel.

Szwecja	2n = 14	A. Löve, 1954
Dania	2n = 14	S. Erlandsson, 1946
Austria	2n = 14	E. Tschermak-Woess, 1948

Materiały z Ogrodów Botanicznych oraz inne o nie zidentyfikowanym pochodzeniu

—	2n = 14	A. J. Hendricks, 1957
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Alisma triviale Pursh.

Ameryka Pn.		
New Hampshire	2n = 14	W. V. Brown, 1946
Grafton Co.		

Alisma subcordatum Raf.

Ameryka Pn.		
Colorado	2n = 28	W. V. Brown, 1946
California	2n = 28	C. B. Heiser i T. W. Whitaker, 1948
Virginia	n = 7	J. T. Baldwin i B. M. Speese, 1955
	2n = 14	

Alisma canaliculatum A. Br. and Bouché

—	2n = 28	A. J. Hendricks, 1957
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Tabela 2 — Table 2

Alisma plantago-aquatica L., *Alisma lanceolatum* With. *Alisma plantago-aquatica* × *Alisma lanceolatum*: wykaz stanowisk*. List of habitats

Lp.	Stanowisko i lata prowadzonych obserwacji Habitats and years of observation	Nr populacji — Strain N.			Szer. geogr. pn. N. Lat.	Dług. geogr. wsch. E. Long.
		<i>A. plantago-aquatica</i>	<i>A. plantago-aquatica</i> × <i>A. lanceolatum</i>	<i>A. lanceolatum</i>		
1	Kośne Hamry k. Zako-panego (1960)	124			49°18'	19°58'
2	Lasek k. Nowego Targu (1960)	125, 127, 129		126, 128 coll. E. Pogan	49°30'	19°59'
3	Sidzina k. Jordanowa (1956)	4			49°37'	19°46'
4	Maków Podhalański (1959)	70			49°44'	19°41'
5	Zarzyce k. Leńc (1959)	72			49°53'	19°45'
6	Leńcze (1959)	71			49°53'	19°43'
7	Wieliczka (1959)	42			49°58'	20°4'
8	Szarów (1959)	56			50°1'	20°17'
9	Staniątki (1959—1960)	57		120 (d) coll. R. Izma- iłow	50°1'	20°13'
10	Łąźnie k. Niepołomic (1958)	16			50°3'	20°13'
11	Bochnia (1952)	59			49°58'	20°26'
12	Jadowniki Mokre (1958—1959)	17, 79			49°58'	20°37'
13	Mokrzyska (1958—1960)	75—78, 91— 94, 96, 97, 99—101, 103, 123(a) 122(b, c)	105, 123(b) coll. E. Pogan	19, 95, 98 102, 104 coll. M. Skalińska 113, 122(a, d) coll. K. Turała E. Pogan	50°2'	20°38'
14	Brzesko-Okocim (1960)				49°58'	20°38'
15	Ciężkowice k. Tarnowa (1957)	8			49°47'	20°59'
16	Muszyna (1959)	85			49°21'	20°54'
17	Biecz (1959)	80			49°44'	21°17'
18	Rybnik-Paruszowiec (1958)	21			50°7'	18°34'
19	Gliwice (1956)	6			50°47'	18°42'
20	Goczałkowice k. Cze- chowic (1959)	67			49°57'	18°58'
21	Polanka Wielka k. Oświę- cimia (1958)	29			49°59'	19°20'
22	Kłucze k. Olkusza (1959)	90			50°20'	19°34'

(Tabela 2 c.d.)

Lp.	Stanowisko i lata prowadzonych obserwacji Habitats and years of observation	Nr populacji — Strain N			Szer. geogr. pn. N. Lat.	Dług. geogr. wsch. E. Long.
		<i>A. plantago- -aquatica</i>	<i>A. plantago- -aquatica</i> × <i>A. lanceola- -tum</i>	<i>A. lanceola- -tum</i>		
23	Kraków-Bieżanów (1957—1960)	9, 110, 112	121(e) coll. E. Pogan	55, 111, 121 (a, b, c) coll. E. Pogan	50°1'	20°2'
24	Kraków-Ludwinów (1958)	32			50°3'	19°57'
25	Kraków-Pychowice (1958)	33			50°3'	19°55'
26	Kraków-Płaszów (1958)	34			50°3'	20°0'
27	Kraków-Pleszów (1959)	41			50°5'	20°5'
28	Kraków-Wieczysta (1958)	30			50°5'	20°0'
29	Kraków-Zakrzówek (1959)	68			50°3'	20°6'
30	Kraków-Mogiła (1960)	106, 107, 108, 109			50°4'	20°5'
31	Kraków-Swoszowice (1958)	27			49°58'	19°58'
32	Mydlniki (1958)	25			50°6'	19°51'
33	Owczary k. Buska (1957)	10			50°27'	20°47'
34	Szczaworyż k. Buska (1957)	11			50°27'	20°49'
35	Busko (1960)	115		114 coll. R. Czapi- pik	50°28'	20°46'
36	Sobków n. Nidą (1959)	58			50°42'	20°27'
37	Dęba-Rozalin k. Huty Komorowskiej (1959)	84			50°26'	21°48'
38	Huta Komorowska (1959—1960)	141, 142		61, 138—140, 143—145 coll. Z. Trela	50°24'	21°47'
39	Grębów k. Sandomierza (1959)	54			50°37'	21°50'
40	Chwałowice k. Zawichostu (1959)	60(a, c)		60(b) coll. Z. Trela	50°48'	21°50'
41	Lublin (4 różne stano- wiska) (1959)	43, 44, 52, 53			51°15'	22°35'
42	Dorohucza (1959)	45, 46			51°10'	23°2'
43	Stręczyn (1959)	47			51°15'	23°8'
	Pojezierze Łęczyńsko- Włodawskie					
44	Jezioro Czarne (1959)	48, 49				
45	Jezioro Białeckie (1959)	50			51°35'	23°8'
46	Jezioro Białe (1949)	51				
47	Tomaszów Mazowiecki (Niebieskie Źródła) (1959)	40			51°35'	20°0'
48	Warszawa (Wał Miedze- szyński) (1959)	74			52°15'	21°0'
49	Toruń (1960)	135		132—134, 136, 137 coll. E. Pogan	53°0'	18°36'

(Tabela 2 c.d.)

Lp.	Stanowisko i lata prowadzonych obserwacji Habitats and years of observation	Nr populacji — Strain No.			Szer. geogr. pn. N. Lat.	Dług. geogr. wsch. E. Long.
		<i>A. plantago- -aquatica</i>	<i>A. plantago- -aquatica</i> × <i>A. lanceola- -tum</i>	<i>A. lanceola- -tum</i>		
50	Jezioro Skępskie (1960)	146			52°53'	19°22'
51	Fordon k. Bydgoszczy (1957, 1960)	118, 119		12 coll. R. Czapik	53°15'	18°15'
52	Pojezierze Mazurskie Olsztyn, Jezioro Pod- kówka (1959)	86			53°46'	20°27'
53	Olsztyn, Jezioro Długie (1959)	89				
54	Wybrzeże Bałtyku Wolin (1958)	35			53°50'	14°35'
55	Niechorze, Jezioro Liwia Łuża (1960)	130			54°2'	14°51'
56	Karwia (Błota Karwiń- skie) (2 różne stan.) (1958, 1959)	15, 62, 63			54°48'	18°7'
57	Jastrzębia Góra (1958, 1959)	24, 64			54°48'	18°18'
58	Błota Bielawskie (1959)	65			54°46'	18°17'
59	Władysławowo (1959)	83			54°47'	18°26'
60	Puck (1960)	116			54°45'	18°25'
61	Ogród Botaniczny w Cam- bridge (pochodzenie ma- teriału: Nantes, Francja) (1959)	37		38		

* Wszystkie stanowiska *Alisma lanceolatum* i *A. plantago-aquatica* × *A. lanceolatum* wykryte zostały w toku niniejszej pracy.

lezione zostały na stanowiskach naturalnych w różnych partiach zasięgu tego gatunku typy kariologiczne $2n = 10, 12, 16$.

W obrębie gatunku *A. lanceolatum* zostały poprzednio wykryte trzy typy kariologiczne — $2n = 24, 26, 28$. Liczba $2n = 24$ znaleziona została dotychczas tylko przez Tarnavskiego (1948, wg Löve i Löve, w druku) w materiale pochodzącym z Rumunii. Biotypy $2n = 28$ zostały zidentyfikowane w Szwecji, Anglii, Danii — w bardziej północnych partiach zasięgu *A. lanceolatum*; natomiast biotypy $2n = 26$ zostały wykryte w Danii, w okolicach Wiednia, w Portugalii — bardziej na południe od poprzednich. Jedynie w Danii, w materiale pochodzącym z wyspy Moen, stwierdzone zostało występowanie obydwu biotypów ($n = 14$ — Hagerup 1944, $2n = 26$ — Tschermak-Woess 1948). Należy dodać,

że podana dla *A. lanceolatum* z Ogrodu Botanicznego w Uppsali liczba chromosomów $2n = 26$ przez Löve i Löve (1944) została ustalona dla roślin o nieokreślonym bliżej pochodzeniu.

W związku z tym nasuwa się pytanie, czy typy kariologiczne *A. lanceolatum* $2n = 26$ i $2n = 28$ reprezentują odrębne rasy geograficzne. Odpowiedź na to pytanie mogłaby dać dopiero wszechstronna analiza kariologiczna i morfologiczna populacji *A. lanceolatum* z różnych partii zasięgu tego gatunku, z uwzględnieniem stanowisk przy jego granicach.

Badania kariologiczne rzuciły również pewne światło na zagadnienie genezy *A. lanceolatum*. Zagadnienie to było dyskutowane przez Erlandsona (1946) oraz przez Tschermak-Woess (1948). Erlandsson przypuszcza, że *A. lanceolatum* jest autotetraploidem powstałym w wyniku podwojenia liczby chromosomów *A. plantago-aquatica*. Autor opiera swój pogląd na dotychczasowych danych taksonomicznych, z uwzględnieniem rozmieszczenia geograficznego obydwu gatunków, jak również na ustalonych przez siebie liczbach chromosomów (*A. plantago-aquatica* — $2n = 14$, *A. lanceolatum* — $2n = 28$) oraz na szczegółach ich morfologii.

Natomiast w inny sposób interpretuje pochodzenie *A. lanceolatum* Tschermak-Woess (1948). Autorka przypuszcza, że badane przez nią formy *A. lanceolatum* ($2n = 26$) mogą być pochodzenia allopoliploidalnego. Ich domniemane formy rodzicielskie stanowić mogły typy $2n = 14$, o morfologii chromosomów zupełnie podobnej do określonej przez autorkę dla *A. gramineum* i *A. plantago-aquatica*, oraz typy $2n = 12$, jakie wg wszelkiego prawdopodobieństwa występują w obrębie *A. plantago-aquatica*. Tschermak-Woess podkreśla, że formami rodzicielskimi *A. lanceolatum* nie mogły być współczesne, badane przez autorkę biotypy *A. plantago-aquatica* i *A. gramineum*, ponieważ ich morfologia chromosomów różni się w pewnych szczegółach od morfologii chromosomów *A. lanceolatum*. Krótkie ramię chromosomów nierównoramiennych jest u *A. lanceolatum* niemal tej samej szerokości co ramię dłuższe, natomiast u obydwu pozostałych gatunków jest ono drobnych rozmiarów, niemal punktowate. Zdaniem autorki za allopoloidalnym pochodzeniem *A. lanceolatum* przemawiają również i pewne cechy morfologiczne tego gatunku, takie jak kształt liści, słabo zgięta szczyłka słupka o pośredniej długości pomiędzy długą i prostą szczyłką *A. plantago-aquatica* a krótką, silnie zgiętą szczyłką *A. gramineum*. Cechą pośrednią byłoby również rozmieszczenie owoców *A. lanceolatum* oraz wykształcenie na ich stronie grzbietowej 1—2 rowków (*A. gramineum* — 2 rowki, *A. plantago-aquatica* — 1 rowek).

Z powyższego wynika, że istnieje znaczna rozbieżność w poglądach autorów na pochodzenie *A. lanceolatum*. Zagadnienie to powinno być rozważane w oparciu o połączone badania cytologiczne i morfologiczne.



Fig. 4. Mapa stanowisk zbadanych kariologicznie populacji z Polski

Map of habitats of strains from Poland, investigated karyologically: $\triangle$ — *Alisma plantago-aquatica* L. ($2n = 14$); $\square$ — *A. lanceolatum* With. ($2n = 26$); $\times$ — *A. plantago-aquatica* L. $\times$ *A. lanceolatum* With. ($2n = 20$) (w miejscach dużego zagęszczenia stanowisk *A. plantago-aquatica* wyznaczony został tylko jeden trójkąt) (the areas in which the habitats of *A. plantago-aquatica* occur in close proximity were marked only by one triangle)

Niniejsze badania nad populacjami gatunków *A. plantago-aquatica* i *A. lanceolatum* z Polski zostały podjęte w tym właśnie celu. W badaniach tych zwróciłam szczególną uwagę na następujące momenty mogące mieć znaczenie ewolucyjne:

Przeanalizowanie możliwie największej liczby populacji *A. plantago-aquatica* i *A. lanceolatum* ze stanowisk naturalnych, zarówno pod względem kariologicznym, jak i morfologicznym. Analiza ta umożliwiłaby

stwierdzenie, czy i na terenie Polski gatunki te wykazują zróżnicowanie kariologiczne; pozwoliłaby ona również na określenie typów morfologicznych biotypów określonych na podstawie kryteriów kariologicznych, co mogłoby rzucić światło na zagadnienie odrębności gatunkowej *A. lanceolatum*. Poznanie typu kariologicznego *A. lanceolatum* mogłoby przyczynić się do wyjaśnienia, czy występujące w obrębie tego gatunku biotypy $2n = 26, 28$ mają charakter ras geograficznych.

Przeprowadzenie u obydwu gatunków dokładnej analizy morfologii chromosomów wydawało się szczególnie ważne dla wyjaśnienia genezy *A. lanceolatum* i ewolucji kariotypu w obrębie rodzaju *Alisma* L.

Praca została wykonana w Zakładzie Anatomii i Cytologii Roślin Uniwersytetu Jagiellońskiego. Kierownikowi Zakładu Pani Profesor Dr M. Skalińskiej wyrażam serdeczne podziękowanie za cenne rady i wskazówki. Dziękuję również za okazaną mi życzliwość i stałe, żywe zainteresowanie tokiem badań.

Panu Profesorowi Dr. B. Pawłowskiemu bardzo dziękuję za uwagi dotyczące pewnych zagadnień poruszanych w morfologicznej części mojej pracy.

MATERIAŁ I METODY

Na terenie Polski występują trzy gatunki rodzaju *Alisma*: *A. plantago-aquatica* L., *A. lanceolatum* With. oraz *A. gramineum* Gmel. *A. plantago-aquatica* jest w naszej florze gatunkiem bardzo pospolitym, natomiast dwa pozostałe, uważane za rzadkie, podawane są dotychczas we florze Polski tylko z nielicznych stanowisk. Zebrany do badań cytologicznych materiał *A. plantago-aquatica* pochodzi z 64 stanowisk z obszaru Polski. Materiał *A. lanceolatum* jest szczuplejszy i obejmuje populacje pochodzące z 10 stanowisk, w których rośliny należące do obydwu zbadanych gatunków występowały w bliskim swoim sąsiedztwie, nierzadko w tych samych kępach (tab. 2, fig. 4).

Materiał do badań kariologicznych stanowiły stożki wzrostu korzeni utrwalanych głównie w terenie. Z utrwalonych roślin pobierano próby kwiatów, owoców i liści do badań morfologicznych i anatomicznych; część roślin hodowano w basenach na terenie Zakładu Anatomii i Cytologii Roślin UJ, część zaś pozostawiono jako materiał zielnikowy dla badań morfologicznych. Utrwalanie korzeni powtarzano w wielu przypadkach na roślinach hodowanych w Zakładzie.

Dla orientacji uwzględniony został również w badaniach cytologicznych materiał *A. plantago-aquatica* i *A. lanceolatum* otrzymany w 1959 r. w postaci nasion z Ogrodu Botanicznego w Cambridge (pochodzenie roślin: Nantes, Francja, 1957).

Podstawę dla badań morfologicznych stanowił własny materiał zielnikowy zbadany uprzednio kariologicznie. Materiał ten został zdeponowany w Zakładzie Anatomii i Cytologii Roślin UJ.

Tabela 3 — Table 3

Alisma lanceolatum With.: zbadane morfologicznie okazy, pochodzące z 6 zielników
Specimens from 6 herbaria studied morphologically

	Stanowisko i siedlisko Habitat	Zebrzał: Coll.:		Materiał ze zbiorów: Herbaria:
1	Hostynice (okolice Hrubieszowa)	Waśniewski	VII.1911	Zielnik Zakładu Botaniki PAN. Kraków
2	Busk	A. Rehman	20.IV.1905	„
3	Przemyśl, rowy łupkowskiej kolei pod Bakończycami	B. Kotula	26.VIII.1876	„
4	Opoka Duża k. Annapola, Lubelszczyzna: łąka nad Senną	D. Fijałkowski	22.VIII.1959	Zielnik Katedry Systematyki i Geografii Roślin Uniw. M. Curie-Skłodowskiej Lublin
5	Santok, woj. Zielona Góra: łąka nad Notecią	M. Kociołek	2.VIII.1950	Zielnik Katedry Systematyki i Geografii Roślin Uniw. Łódzkiego
6	Pabianice k. Łodzi; n. rzeką (egz. niekompletny)	K. Rzydzińska	18.VIII.1949	„
7	Dębina pod Poznaniem: rów	H. Kosińska	14.VIII.1930	Zielnik Katedry Systematyki i Geografii Roślin Uniw. Poznańskiego
8	Jezioro Młyńskie w Prusimiu; pow. Międzychód. Na brzegu wschodnim	I. Dąbska	26.VI.1952	„
9	„Marienwerder” koło Polaszek, pow. sztumski, woj. gdańskie	„Herbarium von Klinggräff”	IX.1855	Zielnik Katedry Systematyki i Geografii Roślin Uniw. M. Kopernika w Toruniu
10	Okręg Elbląg: nad brzegiem Nogatu, przy „Ellerwald”	nieczytelne	19.VIII.1883	„
11	Wrocław: między miastem a „Marienau”, nad brzegiem Odry	nieczytelne	IX.1862	Zielnik Katedry Morfologii i Systematyki Roślin Uniw. Wrocławskiego
12	Wrocław: Rów nad starą drogą, Ołtaszyn	nieczytelne	VII.1877	Zielnik Katedry Morfologii i Systematyki Roślin Uniw. Wrocławskiego
13	Wieś Lubiąż: pow. wołowski, woj. wrocławskie	Kabała	—	„
14	Siedlce, pow. wrocławski	Kabała	7.VIII.1879	„
15	Opatowice, pow. wrocławski	E. Kuźniewski	18.VII.1951	„

Badania morfologiczne zostały rozszerzone na liczne okazy *A. plantago-aquatica* i na zestawione w tabeli 3 okazy *A. lanceolatum*, pochodzące z następujących zbiorów:

1. Zielnik Zakładu Botaniki PAN, Kraków.
2. Zielnik Katedry Systematyki i Geografii Roślin Uniwersytetu Jagiellońskiego, Kraków.
3. Zielnik prywatny Prof. Dr B. Pawłowski, Kraków.
4. Zielnik Katedry Systematyki i Geografii Roślin Uniwersytetu: Marii Curie-Skłodowskiej, Lublin.
5. Zielnik Katedry Systematyki i Geografii Roślin Uniwersytetu Łódzkiego, Łódź.
6. Zielnik Katedry Systematyki i Geografii Roślin Uniwersytetu Poznańskiego, Poznań.
7. Zielnik Katedry Systematyki i Geografii Roślin Uniwersytetu Mikołaja Kopernika, Toruń.
8. Zielnik Katedry Systematyki i Geografii Roślin Uniwersytetu Warszawskiego, Warszawa.
9. Zielnik Katedry Morfologii i Systematyki Roślin Uniwersytetu Wrocławskiego.

Poza tym uwzględnione zostały w badaniach morfologicznych materiały zielnikowe zebrane przez autorkę, a nie zidentyfikowane kariologicznie.

Równocześnie z badaniami wykonywanymi na materiałach zielnikowych prowadzone były przez 4 sezony wegetacyjne obserwacje na materiale żywym, w warunkach naturalnych, jak również na roślinach hodowanych w warunkach wyrównanych.

Kierownikom Zielników, którzy udostępni mi materiały do badań, oraz wszystkim osobom, które wskazały mi stanowiska, dostarczyły żywe rośliny lub nasiona, składam wyrazy podziękowania.

KARIOLOGIA

Badania dotychczasowe

Liczby chromosomów

Z dotychczasowych badań kariologicznych (tabela 1) wynika, że *A. plantago-aquatica* i *A. lanceolatum* wykazują wewnątrzgatunkowe różnicowanie kariologiczne. W materiałach pochodzących ze stanowisk naturalnych zostały stwierdzone dla *A. plantago-aquatica* liczby chromosomów $2n = 10, 12, 14, 16$. Typ kariologiczny $2n = 14$ jest najczęstszy, a reprezentujące go rośliny znalezione zostały zarówno w Europie północnej,

środkowej i południowej, jak również w Ameryce Pn. W obrębie *A. plantago-aquatica* var. *americanum* Hendricks (1957) stwierdził sporadyczne występowanie form tetraploidalnych $2n = 28$ oprócz typowych dla tej odmiany — $2n = 14$.

Typy kariologiczne $2n = 10, 12, 16$ podawane były obok typu $2n = 14$ z różnych partii zasięgu *A. plantago-aquatica*. W materiale ze Szwecji, najpełniej dotychczas opracowanym pod względem kariologicznym, znaleziona została obok $2n = 14$ również liczba $2n = 12$. Podają ją Löve i Löve (1942) dla materiału z Åhus, wzmiankując w swojej późniejszej pracy (1944), że liczbę tę znalazła Blomstrand, oprócz $2n = 14$ (stanowisko nie podane). Palmgren (1943) ustalił $2n = 12$ ($n = 6$) w materiale pochodzącym z Gryft w prowincji Södermanland oraz z Tynderö w Medelpad. Jest faktem godnym uwagi, iż Erlandsson (1946) mając do dyspozycji obszerne materiały pochodzące głównie ze Szwecji (11 stanowisk w Szwecji, 1 — w Danii, 1 — w Finlandii) stwierdził w nich wyłącznie $2n = 14$. Liczba chromosomów $2n = 12$ podawana była przez Tarnavskiego (1948: wg Löve i Löve, w druku) dla materiału z Rumunii. Uprzednio znacznie wcześniej znalazł ją Liehr (1916, str. 183) przypuszczalnie dla materiału z Niemiec. Obszar Niemiec stanowi drugie centrum występowania biotypów aneuploidalnych. Wulff (1939) ustalił dla materiału pochodzącego z Trönnelsee w pobliżu Kilonii liczbę chromosomów $2n = 10$. Ponieważ jednak liczba ta nie została potwierdzona w latach późniejszych, Wulff (1950) przebadał materiały ponownie zebrane z tego samego stanowiska i chociaż nie znalazł w nich typów kariologicznych $2n = 10$, to jednak po powtórным przebadaniu starych preparatów stwierdził, iż liczba ta jest z całą pewnością ustalona poprawnie. Tę samą liczbę podał dla *A. plantago-aquatica* prócz Wulffa tylko Wewetzer (1956, wg Löve i Löve, w druku). W nowo zebranym materiale z okolic Kilonii Wulff (1950) znalazł obok biotypów $2n = 14$ ($n = 7$) nowe o nie podawanej dotychczas liczbie chromosomów $2n = 16$ ($n = 8$).

A. lanceolatum, podobnie jak *A. plantago-aquatica*, wykazuje zróżnicowanie kariologiczne. W obrębie tego gatunku stwierdzone zostało występowanie typów kariologicznych $2n = 24, 26, 28$.

Pierwsze dane dotyczące kariologii *A. lanceolatum* mamy w pracy Hagerupa (1944), w której autor podaje dla materiału z wyspy Moen w Danii $n = 14$. Tę samą liczbę chromosomów ($2n = 28$) znalazł Erlandsson (1946) w materiale z 1 stanowiska w Danii i 2 stanowisk w Szwecji, jak również w materiale z Ogrodu Botanicznego w Uppsali (pochodzenie materiału nie podane), uprzednio badanym przez Löve i Löve (1944). Autorzy ci ustalili natomiast na materiale z tego samego Ogrodu Bot. nową nie podawaną poprzednio dla *A. lanceolatum* liczbę

$2n=26$. Dla materiału z Anglii Priestley (1953) podaje $2n=28$. Liczba $2n=26$ podawana przez Löve i Löve (1942) została potem potwierdzona przez innych badaczy. Znaleziona ona została przez Tschermak-Woess (1948) w materiale z 6 stanowisk w okolicach Wiednia, jak również u 10 roślin stanowiących potomstwo roślin z Ogródu Botanicznego w Kopenhadze (pochodzących z wyspy Moen, a być może i z innych stanowisk w Danii). Liczbę chromosomów $2n=26$ podają także de Castro i Noronha-Wagner (1950, wg Löve i Löve, w druku) dla materiału z Portugalii.

Biotypy $2n=28$ zostały znalezione w bardziej północnych partiach zasięgu gatunku *A. lanceolatum*, zaś biotypy $2n=26$ bardziej na południe od nich. Tylko na terenie Danii stwierdzone zostało występowanie obydwu typów kariologicznych. Typy kariologiczne $2n=26, 28$ wydają się stanowić odrębne rasy geograficzne.

Liczba $2n=24$ ustalona została dla *A. lanceolatum* tylko przez Tarnavskiego (wg Löve i Löve, w druku) dla materiału z Rumunii i dla tego nie można na tej podstawie wyciągać ogólniejszych wniosków, dotyczących charakteru tych biotypów.

Z uwagi na fakt, iż ustalenie liczby chromosomów gatunków rodzaju *Alisma* przedstawia znaczne trudności, niektórzy autorzy poddają w wątpliwość poprawność liczb nie stanowiących wielokrotności liczby podstawowej $x=7$, nie wykluczając jednak możliwości występowania w obrębie tego rodzaju gatunków o zróżnicowaniu aneuploidalnym (Baldwin i Speese 1955).

Morfologia chromosomów

W pracach wcześniejszych z zakresu kariologii gatunków rodzaju *Alisma* najczęściej brak danych dotyczących morfologii chromosomów, albo dane te są fragmentaryczne, oparte często na kryteriach subiektywnych. Zamieszczone w niektórych pracach ryciny są trudne do zanalizowania, a tym samym trudno porównywalne.

Ważne uwagi dotyczące morfologii chromosomów zamieszczone są w pracy Erlandssona (1946). Uwagi te, jak również odpowiednie liczne ryciny płytek metafazowych trzech występujących w Europie gatunków rodzaju *Alisma*, pozwalają jednak jedynie na ogólne zorientowanie się w typie chromosomów tych gatunków. Autor wyróżnia u *A. plantago-aquatica* ($2n=14$) trzy typy chromosomów: długie, średnie i krótkie. Chromosomy długie mają centromer zlokalizowany medialnie lub subterminalnie. We wszystkich płytkach autor obserwował tylko jeden chromosom szczególnie długi. W jednej płytce metafazowej widoczne były 2 chromosomy z trabantami. Chromosomy *A. lanceolatum* są również trzech typów i tego samego kształtu co u *A. plantago-aquatica*.

Wnikliwą analizę morfologii chromosomów *A. plantago-aquatica*, *A. lanceolatum* i *A. gramineum* przeprowadziła Tschermak-Woess (1948) na rozmazach ze stożków wzrostu korzeni i pędów uprzednio poddawanych działaniu kolchicyny dla przykurczenia chromosomów. W materiałach pochodzących z okolic Wiednia autorka ta stwierdziła w płytkach metafazowych *A. plantago-aquatica* ($2n=14$) i *A. gramineum* ($2n=14$):

- 5 par chromosomów równoramiennych (w czym jedna para chromosomów szczególnie długich, pozostałe $\pm$ jednakowej średniej wielkości);
- 2 pary chromosomów nierównoramiennych, krótkich; jedno ramię tych chromosomów jest drobne, kuliste, niemal punktowate; jedna z tych dwu par chromosomów nierównoramiennych jest nieco dłuższa, z trabantem na krótszym ramieniu.

W płytkach metafazowych *A. lanceolatum* ($2n=26$) Tschermak-Woess znalazła: 22 chromosomy równoramienne oraz 4 chromosomy nierównoramienne, z których dwa mają trabanty na krótszym ramieniu; trabanty *A. lanceolatum* są większe niż u obydwu poprzednio omówionych gatunków.

Morfologia chromosomów okazów *A. plantago-aquatica* i *A. lanceolatum* pochodzących z Danii jest zdaniem Tschermak-Woess bardzo podobna do morfologii chromosomów odpowiednich gatunków z okolic Wiednia, a różnica polega tylko na wielkości trabantów: w materiale z Danii są one większe niż w materiale z okolic Wiednia.

Dla zanalizowania i porównania kariotypów $2n=10, 14, 16$ Wulff (1950) ustalał w nich zarówno w mitozie, jak i mejozie liczbę najmniejszych chromosomów, określonych przez niego jako chromosomy „G”. Typ $2n=14$ ma dwa chromosomy „G” (w mejozie jeden mały biwalent „G”); typ hypodiploidalny — $2n=10$ nie ma w ogóle tych chromosomów i nie ma jednej pary chromosomów równoramiennych, zaś typ hyperdiploidalny ma cztery chromosomy „G” (w mejozie dwa biwalenty „G”). Genezę tego śródgatunkowego zróżnicowania kariologicznego *A. plantago-aquatica* Wulff tłumaczy zjawiskami eliminacji albo podwojenia pewnych typów chromosomów.

Oleson (1941) zbadała liczbę chromosomów i ich morfologię u okazów *A. plantago* L. pochodzących z Ameryki Pn. Wyróżniła ona u nich trzy typy chromosomów: długie, średnie i krótkie. Spośród czterech chromosomów długich dwa mają centromer medialny, zaś dwa subterminalny. Pozostałych 10 chromosomów ma centromer subterminalny: sześć z nich jest średniej długości, zaś cztery są krótkie. W nielicznych płytkach autorka obserwowała obecność trabanta na krótkim ramieniu jednego z krótkich chromosomów.

Zupełnie inny typ morfologii chromosomów, wykazujący jednak daleko idące podobieństwa do morfologii chromosomów *A. plantago-aquatica* (opracowanej przez Tschermak-Woess), został stwierdzony przez Browna (1946) dla amerykańskiego gatunku *A. triviale* Pursh ($2n=14$), blisko spokrewnionego z *A. plantago-aquatica*. Autor wyodrębnił tu 10 chromosomów z centromerem medialnym oraz 4 chromosomy z centromerem subterminalnym. Długości chromosomów mieszczą się w granicach 8—22 μ . Brown podkreśla, że tetraploidalny gatunek amerykański *A. subcordatum* Raf. ($2n=28$) zdaje się mieć dwa kompleksy chromosomów, z których każdy jest bardzo podobny z kształtu i długości chromosomów do kompleksu chromosomowego *A. triviale*.

Na materiale diploidalnego biotypu *A. subcordatum* ($2n=14$), uprzednio traktowanym dla przykurczenia chromosomów paradichlorobenzenem, Baldwin i Speese (1955) stwierdzili daleko idące podobieństwo w morfologii chromosomów tego biotypu oraz *A. plantago-aquatica* i *A. gramineum*, zinterpretowanej przez Tschermak-Woess. Podkreślają oni jednak u *A. subcordatum* brak trabantów, dających się zidentyfikować u poprzednich dwu gatunków. Z idiogramu jak również z zamieszczonej w pracy ryciny płytki metafazowej wynika, o czym autorzy nie wzmiankują, że w kompleksie chromosomowym *A. subcordatum* występuje para chromosomów z centromerem submedialnym. Tego typu chromosomów nie obserwowała Tschermak-Woess w materiałach przez siebie badanych.

Badania własne

Metodyka badań.

Liczby chromosomów zostały ustalone na płytkach metafazowych w stożkach wzrostu korzeni roślin zebranych ze stanowisk naturalnych (tabela 2). W nielicznych przypadkach badania te zostały przeprowadzone na stożkach wzrostu korzeni siewek wyhodowanych z nasion.

Dla uzyskania potrzebnego do badań cytologicznych materiału siewek zostały przeprowadzone próby kiełkowania nasion w różnych warunkach doświadczalnych. Najlepsze rezultaty osiągnęłam po wypreparowaniu nasion z owocni albo też po mechanicznym uszkodzeniu owocni. W obydwu przypadkach nasiona wysiane na szalkach Petriego na bibule filtracyjnej kiełkowały już w następnym dniu po wysiewie. Dobre rezultaty dawało również wysiewanie nasion w piasku pod warstewką wody. W 2—4 tygodni po wysianiu ukazywał się liścień. Wyniki te są różne od otrzymanych poprzednio przez Wulffa (1950), który w doświadczeniach swoich stwierdził, że nasiona *Alisma plantago-aquatica* kiełkowały dopiero w drugim roku po wysianiu.

Stożki wzrostu korzeni do badań kariologicznych utrwalalam zwykle w terenie; często powtarzałam utrwalanie na materiale hodowanym (w Zakładzie). Stosowano zawsze utrwalacz Navashina rozcieńczony wodą destylowaną w stosunku 1 : 1. Skrawki parafinowe grubości 18μ barwiono fioletem genejanowym wg Newtona.

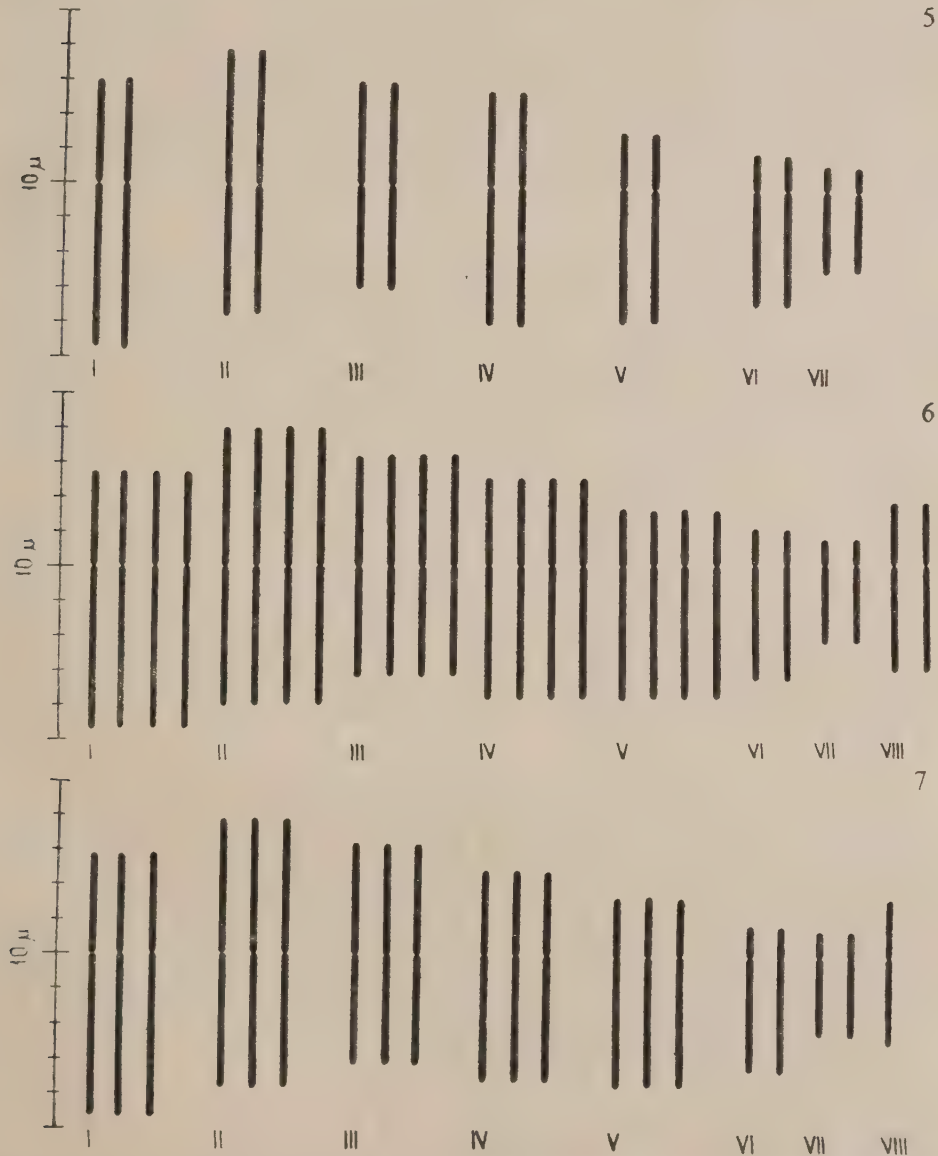


Fig. 5—7. Idiogramy. Idiograms: 5 — *Alisma plantago-aquatica* L. ($2n = 14$); 6 — *A. lanceolatum* With. ($2n = 26$); 7 — *A. plantago-aquatica* L. $\times$ *A. lanceolatum* With. ($2n = 20$)

Chromosomy *Alisma* są długie, wykazują tendencję do skupiania się w płytkach metafazowych zarówno w mitozie, co już podkreślali Wulff (1950) i Erlandsson (1946), jak i w mejozie Hagerup (1944). Z tego powodu obrazy cytologiczne płytek są trudne do interpretacji. Niektórzy autorzy podkreślali, że dobre rezultaty w ustaleniu liczby chromosomów i ich morfologii dawało traktowanie obiektów substancjami powodującymi przykurczanie się chromosomów, takimi jak kolchicina (Tschermak-Woess 1948) lub paradichlorobenzen (Baldwin i Speese 1955). Metoda ta, jakkolwiek bardzo pomocna przy określaniu liczby chromosomów, wydaje się mimo wszystko przedstawiać pewne niebezpieczeństwo w analizie ich morfologii, zwłaszcza wtedy gdy różnice długości chromosomów kompleksu, czy też różnice długości ramion chromosomów są nieznaczne.

Ustalenie liczby chromosomów na materiale z Polski oparte zostało na analizie obszernego materiału z różnych stanowisk. Z selek płytek metafazowych wybrane zostały dla każdej rośliny 3—10 płytek o dobrze rozdzielonych, silnie zespiralizowanych chromosomach, nie następujące trudności w określeniu ich liczby.

Badania nad morfologią chromosomów przeprowadziłam na płytkach metafazowych o uprzednio określonej liczbie chromosomów. Płytki te rysowane były spod mikroskopu w powiększeniu $3000\times$ i na podstawie rysunków ustalone zostały długości ramion chromosomów. Średnie wartości tych pomiarów, zestawione w tabeli 4, posłużyły do wykonania idioграмów (fig. 5, 6, 7). Zidentyfikowanie par chromosomów homologicznych *Alisma* przedstawia znaczne trudności z uwagi na niezbyt duże różnice wielkości długich chromosomów kompleksu, jak również na nieznaczne różnice długości ich ramion.

„Wzorzec” różnych typów morfologicznych chromosomów *Alisma* uzyskano na podstawie obserwacji przeprowadzonych na anafazowych grupach chromosomów, widocznych niekiedy na poprzecznych przekrojach wierzchołka wzrostu korzenia, w różnicujących się członach naczyń. W wielkich komórkach inicjalnych członów naczyń chromosomy grupy anafazowej mają niekiedy ramiona silnie odchylone od długiej osi wrzeciona, przy tym skutek ruchu anafazowego są one wyprostowane. Analiza takiej grupy anafazowej chromosomów, oglądanej szczególnie w widoku od strony bieguna, pozwala na zorientowanie się w typach chromosomów oraz w stosunkach długości ich ramion.

Liczby chromosomów i ich morfologia

We wszystkich roślinach *A. plantago-aquatica* z Polski znaleziona została tylko jedna liczba chromosomów: $2n = 14$ (fig. 13a, 13b). Pomimo przeanalizowania obszernego materiału z 64 stanowisk nie znaleziono

typów aneuploidalnych podawanych z innych obszarów zasięgu tego gatunku. Również i u roślin z grodu Botanicznego w Cambridge (pochodzących z Nantes) znaleziono $2n = 14$.

Morfologia chromosomów *A. plantago-aquatica* opracowana na materiale z Polski wykazuje w ogólnych zarysach podobieństwo do opisanej dla tego gatunku przez Tschermak-Woess (1948).

Jak widać w idiogramie (fig. 5), wyznaczonym dla materiału z Polski, w diploidalnym kompleksie chromosomowym *A. plantago-aquatica* ($2n = 14$) występują dwie pary chromosomów z centromerem wyraźnie medialnym (II, III). Dwie inne mają centromer submedialny (I, IV); różnice długości ramion każdego z tych chromosomów są wprawdzie nieznaczne, ale w dobrze rozstawionych płytkach metafazowych zawsze dostrzegalne. Jedna para chromosomów ma centromer wyraźnie submedialny (V). Prócz powyższych typów chromosomów w kompleksie *A. plantago-aquatica* występuje: jedna para dłuższych (VI) i jedna para krótszych (VII) chromosomów z centromerem subterminalnym; w niektórych płytkach widoczna jest w tych chromosomach wyraźnie nie barwiąca się strefa centromerowa („przewężenie pierwotne“). Na krótkim ramieniu pary dłuższych chromosomów z centromerem subterminalnym obserwowano w kilku płytkach drobny trabant (nie uwzględniony w idiogramie).

Wszystkie przebadane z Polski okazy *A. lanceolatum* okazały się typami $2n = 26$ (fig. 14a, 14b). Znalezione one zostały w materiałach zarówno z południowego, jak i północnego obszaru Polski i należy przypuszczać, że tylko ten typ kariologiczny *A. lanceolatum* występuje na całym obszarze Polski. Również w materiale siewek — wykiełkowanych z nasion pochodzących z Nantes stwierdzono $2n = 26$. Jest to zgodne z wyrażonym we wstępie przypuszczeniem dotyczącym geograficznego rozgraniczenia biotypów $2n = 26$ i $2n = 28$.

Dokładna analiza kompleksu chromosomowego *A. lanceolatum* — $2n = 26$ (fig. 6) wykazała, że można w nim zidentyfikować wszystkie typy chromosomów, jakie występują u *A. plantago-aquatica*. Z porównania idiogramów obydwu gatunków (fig. 5, 6) wynika, że chromosomy I, II, III, IV, V są reprezentowane u *A. lanceolatum* w liczbie poczwórnej, a więc dwukrotnie większej niż u *A. plantago-aquatica*. Natomiast w liczbie podwójnej, tak samo jak u *A. plantago-aquatica*, występuje tu każdy z obydwu chromosomów z centromerem subterminalnym (VI, VII). Na krótkim ramieniu pary chromosomów, oznaczonych w idiogramie liczbą „VI“, obserwowano w kilku płytkach drobny trabant (nie uwzględniony w idiogramie). Oprócz tych typów chromosomów występuje w płytkach metafazowych *A. lanceolatum* jedna para chromosomów z centromerem submedialnym (VIII), których nie ma u *A. plantago-aquatica*. Zastępuje ona dwie, spośród czterech par chromosomów z centromerem subterminalnym,

które teoretycznie powinnyby występować u formy euploidalnej *Alisma lanceolatum* ($2n=28$). Jest możliwe, iż ten nowy typ chromosomów powstał w wyniku zmian strukturalnych, jakie objęły niehomologiczne chromosomy z centromerem subterminalnym. Zmiany te polegały przypuszczalnie na nierównej translokacji, która zaszła pomiędzy chromosomami VI i VII. Poprzedzającą translokację fragmentacja chromosomów otwarła możliwości dla wymiany ich odcinków. Powstanie chromosomu VIII możemy wyobrazić sobie zatem w sposób następujący:

Dłuższe ramię chromosomu VI zostało rozdzielone wskutek fragmentacji w pobliżu centromeru na dwa fragmenty: krótszy — centryczny, oraz dłuższy — acentryczny. Zachodząca równocześnie fragmentacja krótkiego ramienia chromosomu VII spowodowała odszczepienie się od niego drobnego acentrycznego fragmentu. Krótkie ramię chromosomu VII, pozbawione końcowego odcinka, uległo następnie zespoleniu z fragmentem acentrycznym chromosomu VI i w wyniku tej translokacji powstał chromosom VIII z centromerem submedialnym. Natomiast acentryczny fragment chromosomu VII oraz fragment centryczny chromosomu VI mogły ulec eliminacji, być może również po ich uprzednim zespoleniu się.

Teoretycznie możliwa jest również i druga ewentualność, mianowicie taka, iż fragmentacja ramion dokonała się w obrębie dłuższego ramienia chromosomu VII i krótkiego ramienia chromosomu VI; dalszy proces przebiegałby analogicznie jak w przypadku poprzednio opisanym.

Wyjaśnienie tego zagadnienia mogłaby dać jednak dopiero analiza przebiegu procesu mejozy u mieszańców. Projekt takich badań jest realny wobec znalezienia w toku niniejszej pracy organizmów mieszańcowych.

W zanalizowanym pod względem kariologicznym materiale z Polski znalezione zostały w 1960 r. trzy rośliny (nr 105, 121d, 123b), których liczba chromosomów różniła się od ustalonej dla obydwu gatunków. Rośliny te pochodziły z dwu stanowisk (tab. 2), z środowisk silnie zaburzonych przez człowieka. Na stanowiskach tych występowały również łącznie populacje *A. plantago-aquatica* i *A. lanceolatum*. W płytkach metafazowych tych trzech roślin liczone $2n=20$. Ponieważ sygnalizowane było z innych krajów występowanie typów aneuploidalnych, a poza tym istniało teoretyczne prawdopodobieństwo sporadycznego występowania triploidalnych form *A. plantago-aquatica* ($2n=21$) było bardzo ważne sprawdzenie tych wyników. Dlatego badania zostały powtórzone na nowo utrwalonym w następnym sezonie wegetacyjnym materiale stożków wzrostu jedynej z trzech roślin utrzymanej przy życiu (nr 105), hodowanej w Zakładzie Anatomii i Cytologii Roślin UJ. Zbadany też został cały dostępny materiał korzonków utrwalonych poprzednio, a pochodzący z dwu pozostałych roślin, zachowanych jako materiał zielnikowy.

Powtórne badania potwierdziły pierwotny wynik. Okazało się z całą

Tabela 4—Table 4

Alisma plantago-aquatica, *A. lanceolatum*, *A. plantago-aquatica* *A. lanceolatum*: długość chromosomów w μ

Length of chromosomes in μ

	<i>Alisma plantago-aquatica</i>	<i>Alisma lanceolatum</i>	<i>Alisma plantago-aquatica</i> $\times$ $\times$ <i>A. lanceolatum</i>
Typ chromosomów I			
liczba w płytce	2	4	3
długość ramion	4,73	4,76	4,70
	3,03	2,73	2,90
dł. całego chrom.	7,76	7,49	7,60
Typ chromosomów II			
liczba w płytce	2	4	3
długość ramion	3,86	4,03	3,96
	3,83	4,00	4,00
dł. całego chrom.	7,69	8,03	7,96
Typ chromosomów III			
liczba w płytce	2	4	3
długość ramion	3,00	3,23	3,16
	3,00	3,23	3,16
dł. całego chrom.	6,00	6,46	6,32
Typ chromosomów IV			
liczba w płytce	2	4	3
długość ramion	4,00	3,93	3,66
	2,80	2,50	2,46
dł. całego chrom.	6,80	6,43	6,12
Typ chromosomów V			
liczba w płytce	2	4	3
długość ramion	3,96	3,90	3,86
	1,66	1,63	1,76
dł. całego chrom.	5,62	5,53	5,62
Typ chromosomów VI			
liczba w płytce	2	2	2
długość ramion	3,35	3,40	3,33
	0,95	1,00	0,90
dł. całego chrom.	4,30	4,40	4,23
Typ chromosomów VII			
liczba w płytce	2	2	2
długość ramion	2,46	2,30	2,33
	0,70	0,66	0,67
dł. całego chrom.	3,16	2,96	3,00
Typ chromosomów VIII			
liczba w płytce	—	2	1
długość ramion	—	3,06	2,50
	—	1,83	1,73
dł. całego chrom.	—	4,89	4,23

pewnością, że w płytkach metafazowych tych roślin występuje dokładnie 20 chromosomów (fig. 15a, 15b, 16a, 16b). Już więc sama liczba chromosomów nasuwała przypuszczenie, iż mamy tu do czynienia z roślinami pochodzenia mieszańcowego.

Przypuszczenie to zostało potwierdzone wynikami analizy morfologii chromosomów domniemanych mieszańców (fig. 7). Jak to było teoretycznie do przewidzenia, chromosomy I, II, III, IV, V występują w płytkach 20-chromosomowych w liczbie potrójnej (po jednym z *A. plantago-aquatica* i po dwa z *A. lanceolatum*); oprócz nich daje się stwierdzić obecność jednej pary chromosomów dłuższych VI i jednej pary chromosomów krótszych VII, z centromerem subterminalnym, pochodzących w jednakowej liczbie od obu form rodzicielskich, a ponadto jeden chromosom z centromerem submedialnym VIII wprowadzony do kompleksu przez *A. lanceolatum* (tab. 4).

Wynik analizy cytologicznej roślin, mających 20 chromosomów jako liczbę somatyczną, pozwolił zatem na bezsporne zidentyfikowanie ich jako mieszańców pomiędzy *A. plantago-aquatica* i *A. lanceolatum*.

Znalezienie mieszańców w materiale z Polski było dla mnie zupełnie nieoczekiwane, gdyż nie spotkałam w literaturze doniesień o ich występowaniu w warunkach naturalnych. Wręcz przeciwnie, Samuelsson (1932, str. 23) stwierdził, że „In den Fällen wo ich *A. lanceolatum* in der Natur studieren konnte, habe ich vergeblich nach Übergangsformen oder Bastarden gesucht, obgleich die Art wenigstens an ein paar Stellen mit *A. plantago-aquatica* zusammen auftrat“. Samuelsson (1932, str. 4) nie wyklucza jednak możliwości ich występowania.

Brak mieszańców w naturze między tymi gatunkami podkreśla również Priestley (1953).

MORFOLOGIA

Morfologia *A. plantago-aquatica* (fig. 33—38) i *A. lanceolatum* (fig. 39—41, 43)

W dotychczasowej literaturze botanicznej mamy szereg bardzo wartościowych publikacji dotyczących systematyki rodzaju *Alisma* L.

Niektóre z tych prac stanowią wynik długoletnich badań, opartych na materiałach pochodzących z całego świata. Jeżeli mimo tego zostały podjęte w toku niniejszych badań obserwacje nad morfologią *A. plantago-aquatica* i *A. lanceolatum* z Polski, to uczyniłam to z uwagi na stwierdzony przez kariologów fakt występowania w obrębie obydwu gatunków zróżnicowania kariologicznego. Być może, że opracowanie typu morfologicznego określonych typów kariologicznych przyniesie wyjaśnienie

pewnych rozbieżności zaznaczających się w diagnozach tych taxa podawanych przez różnych autorów. Podjęcie analizy morfologicznej było konieczne z uwagi na wyrażone we wstępie przypuszczenie zróżnicowania *A. lanceolatum* na dwie rasy geograficzne.

A. plantago-aquatica i *A. lanceolatum* są roślinami trwałymi o kłaczu silnie bulwiasto zgrubiałym. Łodyga 2 lub więcej razy dłuższa niż liście, prosto wzniesiona, u *A. plantago-aquatica* 10—100 (140) cm, u *Alisma lanceolatum* 10—90 (100) cm długa, mniej więcej do połowy nierozgałęziona, nieulistniona, wyżej rozgałęziona okółkowo. Kwiatestan stopniowo zwężający się ku górze, piramidalny, typu wiechy. Liczba okółków kwiatostanu u *A. plantago-aquatica* 3—8 (9), u *A. lanceolatum* (2) 3—6 (7). Szykulki kwiatowe delikatne 1—2,5 (3) cm długie. Liście przykwiatowe lancetowate. Blaszki liściowe u obydwu gatunków wykazują znaczną zmienność w zależności od wielkości osobnika, jego stadium rozwojowego, położenia liści w roziecie oraz charakteru środowiska, w jakim żyje roślina i oczywiście od charakteru genotypowego rośliny. Już najstarszym monografem rodzaju *Alisma* znany był fakt, iż liście gatunków *Alisma*, podobnie zresztą jak liście licznych innych gatunków roślin wodnych, wykazują znaczną plastyczność budowy morfologicznej, a opisywane na ich podstawie jednostki okazały się modyfikacjami, wykształconymi pod wpływem określonych czynników środowiskowych (poziom wody, szybkość prądu, warunki świetlne). Wysoki poziom wody powoduje wykształcenie się liści znacznie węższych, odpowiadających postacią liściom młodocianym, czy też liściom najstarszym, najwcześniej rozwijającym się z kłacza w danym sezonie wegetacyjnym. Typowe liście *A. plantago-aquatica* są jasnozielone; ogonek liściowy jest niekiedy nieco krótszy, zwykle jednak 1—3 (4) razy tak długi jak blaszka liściowa. Blaszki liściowe jajowate, eliptyczno-jajowate, jajowatolancetowate (2,5) 4,5—15 (25) cm dł., 1,5—7,5 (11) cm szer., zwykle na szczycie zaostrome o podstawie płytkosercowatej, zaokrąglonej lub niekiedy (w liściach dużych) krótkoklinowatej.

Liście *A. lanceolatum* są nieco sinawozielone; ogonek liściowy 1—2,5 (4) razy dłuższy niż blaszka. Blaszki liściowe wąskolancetowate, do jajowatolancetowatych, (2) 4,5—15 (25) cm dł., (0,5) 1—4,5 (6) cm szer., na szczycie zaostrome, u podstawy wydłużenie klinowate (wyjątkowo w liściach dużych ściągnięte).

Dla otrzymania wartości porównywalnych, określających kształt liści, obliczone zostały współczynniki wydłużenia (stosunek długości blaszki liściowej do jej szerokości). Pomiaru przeprowadzone były na materiale zielnikowym, zidentyfikowanym uprzednio kariologicznie, a otrzymane wyniki porównane zostały z wynikami uzyskanymi na nie opracowanym kariologicznie materiale zielnikowym z różnych stanowisk w całej Polsce. Do pomiarów brano tylko liście całkowicie wyrosnięte

Tabela 5 — Table 5

Alisma plantago-aquatica, *Alisma lanceolatum*: współczynnik wydłużenia liści (Ratio length/width of the leaves)

Gatunek Species	Liczba roślin Num- ber of plants	Liczba stanow. Num- ber of habitats	o/o	Klasy — Classes												n	Min—Max	M ± m
				1,5 — 2,1 — 2,7 — 3,3 — 3,9 — 4,5 — 5,1 — 5,7 — 6,3 — 6,9 — 7,5 — 8,1 — 8,7														
				1,5 — 2,1	2,1 — 2,7	2,7 — 3,3	3,3 — 3,9	3,9 — 4,5	4,5 — 5,1	5,1 — 5,7	5,7 — 6,3	6,3 — 6,9	6,9 — 7,5	7,5 — 8,1	8,1 — 8,7			
<i>A. plantago-aquatica</i> (2n = 14)	38	18	33 24 o/o	88 64	17 12									138 100	1,6—3,2	2,33 ± 0,03		
<i>A. plantago-aquatica</i> (okazy nie zbadane cy- tologicznie)*	74	74	108 39,6 o/o	129 47,2	34 12,5	2 0,7								273 100	1,6—3,4	2,25 ± 0,025		
<i>A. lanceolatum</i> (2n = 26)	23	7			1 1,3 8	17 23 22	19 25,7 31	14 19 23	11 14,9 13	4 5,4 15	1 1,3 10	4 5,4 5	1 1,3 5	2 2,7 3	74 100 135	3,2—8,6 4,79 ± 0,13 2,9—8,5 4,96 ± 0,11		
<i>A. lanceolatum</i> (okazy nie zbadane cyto- logicznie *	30	15			5,9	16,3	23	17,1	9,6	11,1	7,4	3,7	3,7	2,2	100			

* Specimens not investigated cytologically

dojrzałych roślin, pomijając w pomiarach liście najstarsze, zbliżone kształtem do liści młodocianych, oraz liście najmłodsze.

Zmienność współczynników wydłużenia liści (tabela 5, fig. 8) u *A. plantago-aquatica* waha się w granicach 1,6—3,4, u *A. lanceolatum* zaś w granicach 2,9—8,6*. Niezbyt typową postać krzywej współczynników wydłużenia liści *A. plantago-aquatica* w materiale karyologicznie

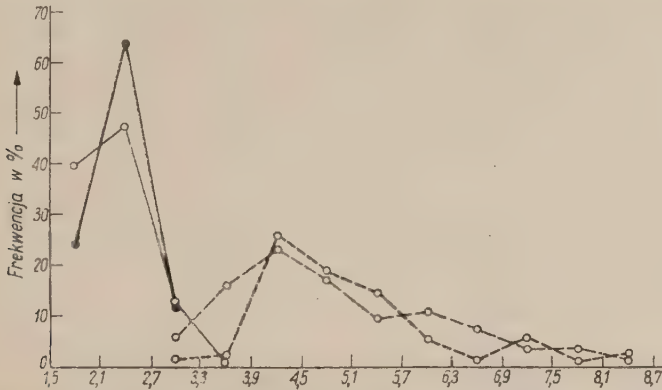


Fig. 8. Wykresy współczynników wydłużenia liści. ——— wyższy szczyt *Alisma plantago-aquatica* L. ($2n = 14$), materiał zielnikowy, zbadany karyologicznie; ——— *A. plantago-aquatica* L., materiał zielnikowy nie zbadany karyologicznie; - - - - - wyższy szczyt *A. lanceolatum* With. ($2n = 26$), materiał zielnikowy zbadany karyologicznie; — · — · — *A. lanceolatum* With., materiał zielnikowy nie zbadany karyologicznie

Graphs of the ratio length/width of the leaves. ——— higher peak *Alisma plantago-aquatica* L. ($2n = 14$), herbarium material investigated karyologically; ——— *A. plantago-aquatica* L., herbarium material not studied karyologically; - - - - - higher peak *A. lanceolatum* With. ($2n = 26$), herbarium material investigated karyologically; — · — · — *A. lanceolatum* With., herbarium material not studied karyologically

nie zidentyfikowanym wynika być może z faktu, iż materiał ten jest wyselekcjonowany w kierunku wyboru okazów mieszczących się w standardowych arkuszach zielnikowych. Zachodzenie na siebie skali zmienności współczynników wydłużenia liści obydwu gatunków w zakresie wartości 2,9—3,4 jest wynikiem występowania w różyczkach liściowych obok liści o wartościach współczynników charakterystycznych tylko dla danego gatunku (*A. plantago-aquatica* — współczynnik wydłużenia liści mniejszy od 2,9; *A. lanceolatum* — współczynnik wydłużenia liści większy od 3,4) liści o wartościach współczynników z zakresu wspólnego dla obydwu gatunków. Współczynniki wydłużenia liści obydwu gatunków różnią się wyraźnie. Różnica ich średnich arytmetycznych jest zna-

* Hendricks (1957) podaje, iż stosunek szer. do dł. liści u *A. lanceolatum* wynosi od $\frac{1}{4}$ do $\frac{1}{3}$.

Tabela 6 — Table 6

Alisma plantago-aquatica, *Alisma lanceolatum*: długość aparatów szparkowych w μ (Length of stomata in μ)

Gatunek Species	Liczba roślin Number of plants	Liczba stanowisk Number of habitats	Klasy — Classes										n	Min — Max	M ± m
			35 — 40	— 45	— 50	— 55	— 60	— 65	— 70	— 75					
<i>A. plantago-aquatica</i> (2n = 14)	11	8	15	120	306	105	4					550	35—55	47,16 ± 0,16	
<i>A. lanceolatum</i> (2n = 26)	11	6			7	87	289	147	19	1		550	45—70	58,29 ± 0,16	

mienna (*A. plantago-aquatica* $M \pm m = 2,33 \pm 0,030$; $2,25 \pm 0,025$; *A. lanceolatum* $M \pm m = 4,79 \pm 0,13$; $4,96 \pm 0,11$). Wstępne obserwacje przeprowadzone na materiale hodowanym w warunkach wyrównanych wykazały, iż cechą najbardziej plastyczną jest wielkość liści; natomiast współczynniki wydłużenia liści mieszczą się w granicach wyznaczonych dla materiału zebranego ze stanowisk naturalnych. Podstawa blaszki liściowej pozostaje u *A. lanceolatum* zawsze klinowata (niekiedy w liściach najszerszych krótkoklinowata). Liście *A. plantago-aquatica* zachowują nasadę zaokrągloną albo płytkosercowatą. Pochodzące ze środowisk wybitnie eutroficznych rośliny *A. plantago-aquatica* o liściach wielkich z podstawą blaszki liściowej krótkoklinowatą wykazały, po przeniesieniu ich w środowisko uboższe w substancje azotowe, tendencję do wykształcenia blaszek liściowych mniejszych i, co charakterystyczne, z nasadą zaokrągloną czy też nawet płytkosercowatą. Przypuszczalnie taki właśnie typ nasady jest uwarunkowany u tego gatunku genetycznie, a specyficzne warunki środowiskowe (eutroficzne) zaburzając tempo wzrostu typowe dla określonych stref liścia maskują tę cechę.

Kształt liści, określony wartością współczynników wydłużenia liści oraz kształtem podstawy blaszki liściowej, jest jedną z cech rozróżniających te dwa gatunki. Różnice w budowie liści obydwu omawianych gatunków zaznaczają się również w wielkości ich aparatów szparkowych. Pomiarzy zostały przeprowadzone na materiale alkoholowym liści. Preparaty wykonane zostały z epidermy górnej powierzchni liści mniej więcej w połowie ich długości. Badaniami zostało objętych 11 roślin każdego gatunku pochodzących głównie ze stanowisk, na których obydwa te gatunki występowały łącznie. Dla każdej rośliny pomierzono długość 50 aparatów szparkowych. Skala zmienności długości aparatów szparkowych (tabela 6, fig. 9) *A. plantago-aquatica* wynosi 35—55 μ , zaś u *Alisma lanceolatum* — 45—70 μ ; (odpowiednie średnie arytmetyczne: *A. plantago-aquatica*: $M \pm m = 47,16 \pm 0,16 \mu$. *A. lanceolatum*: $M \pm m = 58,29 \pm 0,16 \mu$). Otrzymane wyniki są zgodne z przewidywanymi teoretycznie. Podwyższenie stopnia poliploidalności pociąga za sobą i w tym przypadku zwiększenie wielkości komórek. W związku z powyższym należy zaznaczyć, iż długość aparatów szparkowych działek kielicha dla *A. plantago-aquatica* L. var. *Michaletti* (Aschers. et Graebner) Buchenau (taxon odpowiadający *A. plantago-aquatica* (typicum) w ujęciu Samuelssona) wynosi 48,7 μ , odchylenie standardowe 4,7 μ (Hendricks 1957).

Kwiaty obydwu gatunków są obupłciowe promieniste. Okrywa kwiatowa sześciolistkowa jest zróżnicowana na kielich i koronę. Działki kielicha siedzące, szeroko jajowate, brzegiem błoniaste, na szczycie są kształtu kapturkowego. Płatki międzyłgłe w stosunku do działek 2—3 $\times$ tak długie jak działki, po przekwitnięciu szybko opadające: u *A. plantago-aqua-*

lica są one na szczycie ścięte, lub zaokrąglone, drobno karbowane, białe lub biało-fioletowe, (3) 4—6 mm dł., (3) 4—6 mm szer.; u *A. lanceolatum* są one (3) 4—6 mm dł., 4—6 mm szer., odległe ząbkowane, zwykle na szczycie mniej lub bardziej wyraźnie trójkątne; niekiedy tylko środkowa łątka wyciągnięta w trójkątny ząbek. Płatki zwykle bladofioletowe.

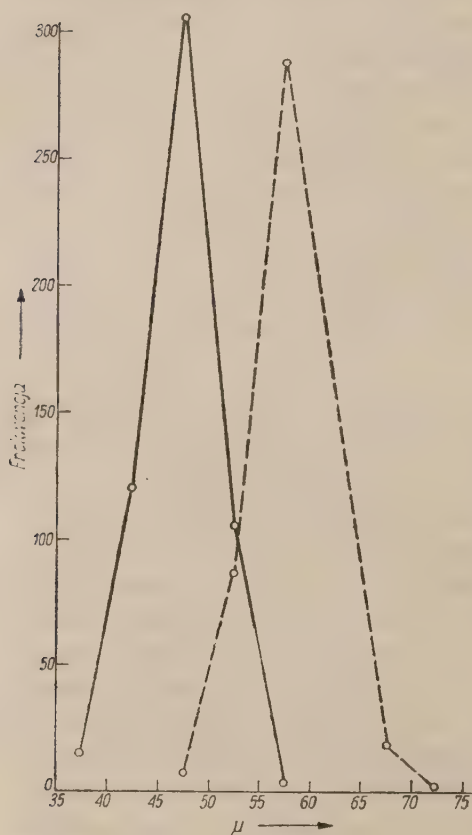


Fig. 9. Wykresy długości aparatów szparkowych w μ (materiał alkoholowy liści). Graphs of the stomata length in μ (alcohol material of leaves). — *Alisma plantago-aquatica* L. ($2n = 14$); - - - *A. lanceolatum* W i t h. ($2n = 26$)

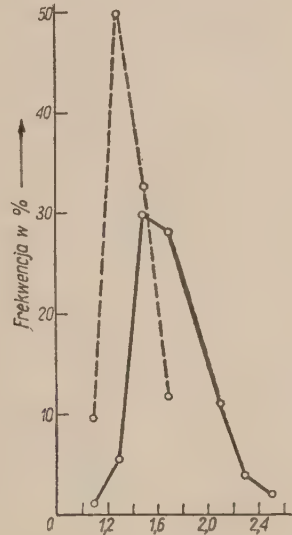
Pręcików 6, $\pm 2 \times$ dłuższych od zalążni: pylniki wolne, wydłużone. Długość pylników pomierzona została dla obydwu gatunków w materiale alkoholowym kwiatów. Po wypreparowywaniu pręcików rysowano ich pylniki w pow. $30 \times$, a otrzymane wartości przeliczane zostały na mm. Skala długości pylników *A. plantago-aquatica* jest szersza (0,7—1,2 mm) niż u *A. lanceolatum* (0,5—0,86 mm). Natomiast skala szerokości pylników obydwu gatunków jest mniej więcej tego samego rzędu (*A. plantago-aquatica* — 0,36—0,8 mm; *A. lanceolatum* — 0,33—0,73 mm).

Współczynniki wydłużenia pylników (stosunek długości pylników do ich szerokości) wyrażają się u *A. plantago-aquatica* wartościami 1,1—2,5 ($M \pm m = 1,73 \pm 0,015$); u *A. lanceolatum* skala ta jest węższa 1—1,7

($M \pm m = 1,39 \pm 0,014$) i przesunięta ku niższym wartościom w skali zmienności *A. plantago-aquatica* (tabela 7, fig. 10).

Ziarna pyłku są kuliste i już na pierwszy rzut oka widoczne są różnice ich wielkości u tych dwu gatunków. Dla porównania wielkości ziarn pyłku pomierzono ich średnicę w materiale zielnikowym (uprzednio rozgotowanym). Do pomiarów wziętych zostało 10 roślin każdego gatunku głównie

Fig. 10. Wykresy współczynników wydłużenia pyl-ników (materiał alkoholowy). Graphs of the ratio length/width of anthers (alcohol material). — length/width of anthers (alcohol material). - - - *A. lanceolatum* With. ($2n = 26$)



ze stanowisk, na których występowały obydwie te gatunki. Dla każdej rośliny mierzono średnicę 50 ziarn pyłku. Ponieważ wyniki z poszczególnych osobników w obrębie gatunku pokrywały się, zostały one zestawione sumarycznie (tab. 8, fig. 11). Skale zmienności średnicy ziarn pyłku obydwu gatunków częściowo na siebie zachodzą; u *A. plantago-aquatica* jest ona jednak przesunięta ku wartościom niższym — $19,5\text{—}30\text{ }\mu$ ($M \pm m = 25,41 \pm 0,09\text{ }\mu$), u *A. lanceolatum* ku wartościom wyższym — $24\text{—}36\text{ }\mu$ ($M \pm m = 30,71 \pm 0,11\text{ }\mu$) zgodnie z liczbami chromosomów tych gatunków.

Słupki u obydwu gatunków liczne ustawione są kolicie na płaskim dnie kwiatowym. Zalążnie nie stykają się ze sobą wewnętrznymi brzegami, pozostawiając w środku wolną przestrzeń. Szyjki słupków proste, lub prawie proste, u *A. plantago-aquatica* są smuklejsze, białawe, nieco dłuższe do znacznie dłuższych od zalążni; znamię drobniutko brodawkowane (fig. 17—25) u *A. lanceolatum* są one krótsze do nieco dłuższych od zalążni, żółtawe o znamieniu grubo brodawkowanym (fig. 28—32).

Z uwagi na fakt, iż cecha ta jest bardzo ważną cechą diagnostyczną, przeprowadzone zostały pomiary długości szyjek słupków na obszernym materiale. Pomiary wykonane zostały na materiale alkoholowym roślin

Tabela 7 — Table 7

Alisma plantago-aquatica, *Alisma lanceolatum*: współczynnik wydłużenia pylników (Ratio of length/width of anthers)

Gatunek Species	Liczba roślin Number of plants	Liczba stanowisk Number of habitats	%	Klasy — Classes										Min — Max	M ± m
				1	1.2	1.4	1.6	1.8	2	2.2	2.4	2.6	n		
<i>A. plantago-aquatica</i> (2n = 14)	19	9	%	3	17	92	87	58	34	12	6	309	1,1—2,5	1,73±0,015	
				1	5,5	29,8	28,1	18,8	11	3,9	1,9	100			
<i>A. lanceolatum</i> (2n = 26)	9	7	%	14	69	44	17					144	1—1,7	1,39±0,014	
				9,7	47,9	30,6	11,8					100			

Tabela 8 — Table 8

Alisma plantago-aquatica, *Alisma lanceolatum*: średnica ziarn pyłku w μ (Diameter of pollen grains in μ)

Gatunek Species	Liczba roślin Number of plants	Liczba stanowisk Number of habitats	Klasy — Classes								n	Min—Max	M ± m
			18 — 21	24	27	30	33	36	39				
<i>A. plantago-aquatica</i> (2n = 14)	10	9	7	101	298	88	6			500	19,5—30	25,41 ± 0,09	
<i>A. lanceolatum</i> (2n = 26)	10	7			30	164	223	74	9	500	24—36	30,71 ± 0,11	

przebadanych kariologicznie, ponadto badania te zostały rozszerzone na nie opracowane kariologicznie materiały zielnikowe z różnych stanowisk w Polsce. Z każdej rośliny zostały wzięte do pomiarów słupkowi trzech kwiatów. Materiały zielnikowe przed rozpreparowaniem kwiatów

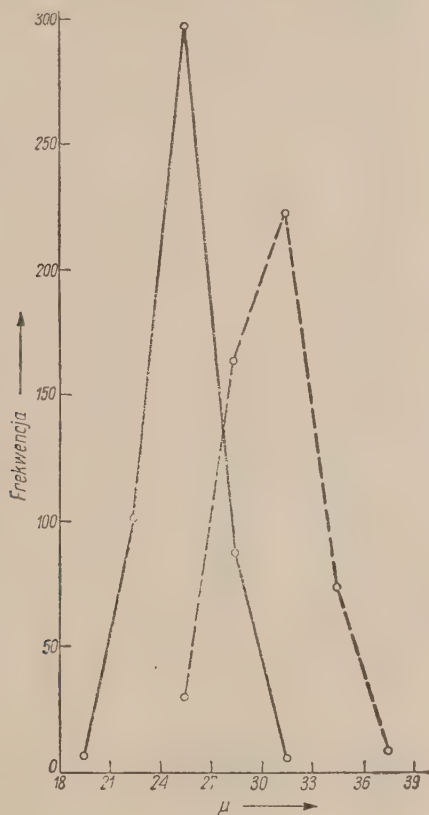


Fig. 11. Wykresy średnicy ziarn pyłku w μ . Graphs of pollen grain diameter in μ . — *Alisma plantago-aquatica* L. ($2n = 14$); - - - *A. lanceolatum* With. ($2n = 26$)

rozgotowano. Wypreparowywane słupki rysowano spod aparatu rysunkowego w pow. $30\times$ i na rysunkach wykonywano pomiary długości szyjek słupków łącznie ze znamieniem. Uzyskane wartości zostały zestawione w tabeli 9 (fig. 12), przy czym osobno uwzględniony został materiał alkoholowy i osobno materiał zielnikowy. Z pomiarów przeprowadzonych na materiale alkoholowym wynika, że skala zmienności długości szyjek słupków u *A. plantago-aquatica* obejmuje wartości z zakresu (0,7) 0,8—1,43 mm ($M \pm m = 1,07 \pm 0,0041$ mm); natomiast u *A. lanceolatum* jest ona przesunięta ku wartościom niższym w zakresie (0,3) 0,4—0,83 mm ($M \pm m = 0,62 \pm 0,0054$ mm). Nieznaczne zachodzenie na siebie skal w zakresie wartości (0,7) 0,8—0,83 mm jest wynikiem sporadycznego występowania niektórych słupków o tej właśnie długości szyjek w słupkowiach obydwu gatunków, których inne słupki mają typową dla każdego z tych gatunków

Tabela 9 — Table 9

Alisma plantago-aquatica, *Alisma lanceolatum*, *Alisma plantago-aquatica* × *A. lanceolatum*: Długość szyjek słupków łącznie ze znamieniem w mm
(Length of styles together with the stigmata in mm)

Gatunek Species	Liczba roślin Number of plants	Liczba stanowisk Number of habitats	%	Klasy — Classes						n	Min—Max	M ± m
				0,3—0,5	0,7	0,9	1,1	1,3	1,5			
<i>A. plantago-aquatica</i> (materiał alkohol.) (2n = 14)*	20	10			53	512	339	23		927	0,8—1,33	1,07 ± 0,0041
<i>A. plantago-aquatica</i> (materiał zielnik. z okazów nie badanych cytologicznie) **	10	10	0/0		5,7	55,3	36,5	2,5		100		
			0/0		40	174	158	10		382	0,7—1,43	1,07 ± 0,0072
<i>A. lanceolatum</i> (materiał alkohol.) (2n = 26)*	10	7		65	307	126				498	0,3—0,83	0,62 ± 0,0054
			0/0	13	61,7	25,3				100		
<i>A. lanceolatum</i> (materiał zielnik. z okazów nie badanych cytologicznie **	7	7		25	171	38				234	0,36—0,83	0,61 ± 0,0067
			0/0	10,7	73,1	16,2				100		
<i>A. plantago-aquatica</i> × <i>A. lanceolatum</i> (materiał alkohol.) (2n = 20)*	2	1		31	16					124	0,6—1,03	0,77 ± 0,011
			0/0	25	62,1	12,9				100		

* alcohol material of specimens investigated cytologically

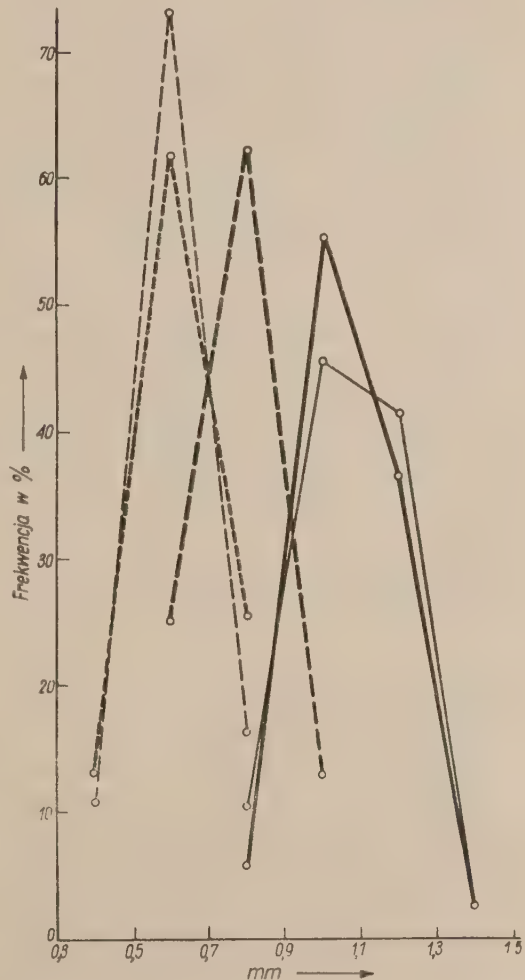
** herbarium material of specimens not investigated cytologically

długość spoza granic powyższego przedziału. Pomiary słupków przeprowadzone na materiale zielnikowym stanowią, z nieistotnymi tylko odchyleniami, potwierdzenie wyników uzyskanych w badaniach przeprowadzonych na materiale alkoholowym.

Owoce jednonasienne silnie spłaszczone o błoniastej owocni u *A. plantago-aquatica* 2—2.5 mm dł., 1,25—1,5 mm szer.; u *A. lanceolatum*: 2—

Fig. 12. Wykresy długości szyjek słupków łącznie ze znamieniem w mm. — wyższy szczyt *Alisma plantago-aquatica* L. ($2n = 14$); materiał alkoholowy zbadany karyologicznie; — *A. plantago-aquatica* L., materiał zielnikowy nie zbadany karyologicznie; - - - - - wyższy szczyt *A. lanceolatum* With. ($2n = 26$), materiał alkoholowy zbadany karyologicznie; - - - - - *A. lanceolatum* With., materiał zielnikowy nie zbadany karyologicznie; - - - *A. plantago-aquatica* L. $\times$ *A. lanceolatum* With. ($2n = 20$), materiał alkoholowy zbadany karyologicznie

Graphs of the length of the styles together with the stigmata in mm. — higher peak *Alisma plantago-aquatica* L. ($2n = 14$), alcohol material investigated karyologically; — *A. plantago-aquatica* L., herbarium material not studied karyologically; - - - - - higher peak *A. lanceolatum* With. ($2n = 26$), alcohol material investigated karyologically; - - - - - *A. lanceolatum* With., herbarium material not studied karyologically; - - - *A. plantago-aquatica* L. $\times$ *A. lanceolatum* With. ($2n = 20$), alcohol material investigated karyologically



2,25 mm dł., 1,25—1,5 mm szer. Na wypukłej stronie grzbietowej owoce obydwu gatunków mają zwykle jeden rowek. Niekiedy występować mogą dwa rowki, ale wówczas żeberko środkowe oddzielające obydwa rowki jest wyższe od żeberków brzeżnych ograniczających je z boku. Samuelsson (1932) podkreśla, że najbardziej uderzającymi cechami *A. lanceolatum* odróżniającymi ten gatunek od *A. plantago-aquatica* są

jego wąskie liście, trójkątne na szczycie płatki i większe brodawki na znamieniu słupka. Autor ten podkreśla również, że *A. lanceolatum* zaczyna kwitnąć wcześniej niż *A. plantago-aquatica*.

Wyniki obserwacji nad morfologią typów kariologicznych *A. plantago-aquatica* ($2n = 14$) oraz *A. lanceolatum* ($2n = 26$) z Polski (rozszerzonych również na rośliny o nieokreślonym uprzednio typie kariologicznym) w odniesieniu do większości cech są zgodne, z niewielkimi odchyleniami, z wynikami badań Samuelssona. Rozbieżność zaznacza się jednak wyraźniej w budowie słupka.

Samuelsson (1932) podaje, iż długość szyjek słupków u *A. plantago-aquatica* wynosi 1—1,25 mm, zaś u *A. lanceolatum* 0,75—1,35 mm. Te same wartości są podawane dla roślin polskich (Szafer, Kulczyński, Pawłowski 1953). Natomiast Glüek (1936) w swojej publikacji „Süsswasser-Flora Mitteleuropas” podkreśla, że szyjki słupków *A. plantago* var. *lanceolatum* są przeciętnie biorąc krótsze niż u var. *latifolium*. Wyniki niniejszych obserwacji wykazują w odniesieniu do tej cechy zgodność z obserwacjami Glüeka, wnosząc przy tym liczbowe ujęcie tych różnic.

Dalszymi cechami pozwalającymi na odróżnienie obydwu gatunków są nie uwzględniane w monografii Samuelssona cechy anatomiczne, dotyczące długości aparatów szparkowych w epidermie liści oraz średnicy ziarn pyłku. Mają one bezsporną wartość diagnostyczną.

Obserwacje wstępne nad morfologią mieszańców *A. plantago-aquatica* ×
× *A. lanceolatum* (fig. 42, 44)

Jak wzmiankowano już uprzednio, na dwu stanowiskach naturalnych znalezione zostały w 1960 r. rośliny, które na podstawie liczby chromosomów $2n = 20$ oraz ich morfologii zostały zidentyfikowane jako rośliny pochodzenia mieszańcowego. Jedna z tych roślin (nr 123b) została po utrwaleniu korzonków do badań kariologicznych zasuszona, z drugiej (nr 105) zasuszono tylko liście i kwiatostan; roślina ta jest nadal w hodowli na terenie Zakładu Anatomii i Cytologii Roślin UJ. Roślina trzecia (nr 121d), przeniesiona po utrwaleniu w stanie niecałkowicie dojrzałym do prywatnego ogrodu, nie rozwinęła kwiatostanu (wskutek powodzi) i tym samym nie nadaje się do obserwacji morfologicznych. Pozostający mi do dyspozycji materiał mieszańców jest bardzo szczupły, a podane poniżej uwagi, dotyczące morfologii mieszańców, mają charakter obserwacji wstępnych. Dalsze badania nad roślinami mieszańcowymi są w toku.

W obserwacjach nad morfologią roślin pochodzenia mieszańcowego zwrócono szczególną uwagę na te cechy, które odróżniają od siebie ga-

tunki rodzicielskie. Pozostałe bowiem cechy mieszczą się w ramach zmienności wspólnych dla obydwu gatunków.

Co się tyczy liści roślin pochodzenia mieszańcowego, to roślina nr 105 wykształciła blaszki liściowe eliptyczne o podstawie krótkoklinowatej. Natomiast roślina nr 123b ma liście eliptycznolancetowate z klinowatą podstawą blaszki liściowej. Materiał liści mieszańców jest zbyt szczupły, aby na jego podstawie przeprowadzić porównanie współczynników wydłużenia liści.

Jest faktem godnym uwagi, że liście rośliny nr 105, hodowanej w Zakładzie, które wykształciły się w sezonie wegetacyjnym 1961, mają płytkosercowatą podstawę blaszki liściowej i są znacznie mniejsze od liści wykształconych w warunkach naturalnych. Być może, że taki typ liści wykształcają rośliny pochodzenia mieszańcowego również w warunkach naturalnych, i może tu kryje się jedna z przyczyn trudności zidentyfikowania ich w naturze.

Tabela 10 — Table 10

Alisma plantago-aquatica × *A. lanceolatum*: długość aparatów szparkowych w μ
Length of stomata in μ

	Klasy — Classes					n	Min—Max	M $\pm$ m
	40 — 45	50	55	60	65			
<i>Alisma plantago-aquatica</i> × <i>A. lanceolatum</i> (Roślina Nr 105) (Plant Nr 105) (2n = 20)	6	30	49	14	1	100	43,3—61,6	51,2 $\pm$ 0,40

W materiale alkoholowym liści rośliny nr 105 pomierzono długość 100 aparatów szparkowych w sposób uprzednio opisany (tabela 10). Skala zmienności długości aparatów szparkowych obejmuje tu wartości 43,3—61,6 μ (M $\pm$ m = 51,2 $\pm$ 0,40 μ).

Płatki roślin pochodzenia mieszańcowego (nr 105, 123b) są 5—6 mm dł., 5—6 mm szer., odlegle ząbkowane, zaokrąglone lub z łatką środkową wyciągniętą w tępy, trójkątny ząbek; barwa płatków bladofioletowa.

Pylniki zredukowane; pyłek zdegenerowany w 100% (roślina 123b) albo tylko w pewnym procencie (u rośliny 105 — do 20% płodny). Średnica żywych ziarn pyłku 21—30 μ (M $\pm$ m = 25,74 $\pm$ 0,35 μ) (tabela 11).

Skala zmienności długości szyjek słupków, pomierzonych dla obydwu roślin, ma wartości pośrednie pomiędzy wartościami wyznaczonymi dla obydwu gatunków rodzicielskich (0,6—1,03 mm; M $\pm$ m = 0,77 $\pm$ 0,011 mm) (tab. 9, fig. 12, 26, 27). Znamiona słupków są grubo brodawkowe, podobnie jak u *A. lanceolatum*.

Owoce (roślina nr 123b) nie wykształcają się; niekiedy ściana zalążni rozrasta się nieco, ale w jej wnętrzu nie tworzy się nasienie.

Jak widać z powyższego omówienia, pewne cechy roślin, zidentyfikowanych na podstawie kryteriów kariologicznych jako mieszańce, mają

Tabela 11 — Table 11

Alisma plantago-aquatica × *Alisma lanceolatum*: średnica ziarn pyłku w μ
Diameter of pollen grains in μ

	Klasy — Classes				n	Min — Max	M ± m
	21 — 24	27	30	33			
<i>Alisma plantago-aquatica</i> × <i>Alisma lanceolatum</i> (Roślina nr 105) (Plant nr 105) (2n = 20)	14	19	16	1	50	21—30	25,74 ± 0,35

charakter pośredni pomiędzy cechami domniemanych form rodzicielskich (długość aparatów szparkowych, kształt płatków, długość szyjek słupków). Budowa znamienia słupka mieszańców wskazuje na dominujący wpływ genomu *A. lanceolatum*. Natomiast skala zmienności średnicy żywych ziarn pyłku (rośliny nr 105) mieści się w obrębie skali zmienności średnicy ziarn pyłku *A. plantago-aquatica*. Z uwagi na budowę kariologiczną mieszańców (triploidy) należy przypuszczać, że przebieg procesu mejozy ulega silnym zakłóceniom i większość ziarn ulega degeneracji; żywotnymi zdają się być tylko ziarna pyłku o liczbie $n = 7$ albo różniacej się od niej tylko nieznacznie.

DYSKUSJA

Zagadnieniem niniejszej pracy było ustalenie wzajemnego stosunku dwóch gatunków rodzaju *Alisma* L., *A. plantago-aquatica* L. i *A. lanceolatum* With. oraz próba wyjaśnienia genezy *A. lanceolatum*.

Zdaniem systematyków *A. plantago-aquatica* i *A. lanceolatum* wykazują bliskie pokrewieństwo, przy czym większość wcześniejszych badaczy przypisywała *A. lanceolatum* rangę systematyczną niższą od gatunku. Natomiast nowsze ujęcia systematyczne (Samuelsson 1922, 1932, 1933; Szafer, Kulczyński, Pawłowski 1953, Hendricks 1957; Dostal 1958) traktują *A. lanceolatum* jako odrębny gatunek; na korzyść tego poglądu przemawiają również przeprowadzone w toku niniejszej pracy badania morfologiczne nad populacjami obydwu gatunków z licznych stanowisk naturalnych w Polsce oraz wstępne obserwacje prze-

prowadzone na materiałach hodowanych w warunkach wyrównanych. Prócz podkreślanych przez systematyków cech diagnostycznych (kształt liści, płatków, typ brodawkowania znamion słupków), na odrębność gatunkową *A. lanceolatum* wskazują nie uwzględniane dotychczas cechy anatomiczne, mające bezsporną wartość diagnostyczną (długość aparatów szparkowych w epidermie liści, średnica ziarn pyłku). Dotąd jedynie Hendricks (1957) uwzględnił dla *A. plantago-aquatica* L. var. *Michaletii* (Aschers. et Graebner) Buchenau dane dotyczące długości aparatów szparkowych ($48,7\mu$; odchylenie standartowe $4,7\mu$).

Na szczególną uwagę zasługują wyniki pomiarów długości szyjek słupków; cecha ta ma dużą wartość diagnostyczną, zwłaszcza w przypadkach krytycznych (Samuelsson 1932). Według Samuelssona skala zmienności długości szyjek słupków *A. plantago-aquatica* obejmuje wartości $1-1\frac{1}{4}$ mm, u *A. lanceolatum* zaś — wartości $\frac{3}{4}-1\frac{1}{3}$ mm. Skala zmienności długości szyjek słupków, wyznaczona na materiałach z Polski (fig. 12), jest w odniesieniu do *A. plantago-aquatica* tylko nieznacznie szersza — (0,7) 0,8—1,43 mm — od podanej przez Samuelssona. Natomiast wyraźna rozbieżność zaznacza się w odniesieniu do *A. lanceolatum*. W materiale z Polski długości szyjek słupków mieszczą się w granicach (0,3) 0,4—0,83 mm. Skala ta obejmuje więc wartości niższe spoza zakresu skali, podawanej dla tego gatunku przez Samuelssona i tylko nieznacznie na nią zachodzi. Szyjki słupków *A. lanceolatum* są więc u okazów z Polski krótsze niż u *A. plantago-aquatica* (fig. 12, 17—25, 28—32). Dane powyższe zgodne są z obserwacjami Glücka (1936) dotyczącymi roślin pochodzących z Europy środkowej i dają ich liczbowe ujęcie.

Za odrębnością gatunkową *A. lanceolatum* przemawia również występowanie pomiędzy obydwoma gatunkami bariery izolacyjnej w postaci całkowitej albo przynajmniej częściowej sterility mieszańców naturalnych. W tym jednak przypadku triploidalny charakter mieszańców i stanowiący jego naturalną konsekwencję nienormalny przebieg procesu mejozy mogą maskować udział innych czynników ewolucyjnych w wykształceniu się tej bariery. Przypuszczalnie dopiero badania nad przebiegiem mejozy u eksperymentalnych mieszańców pomiędzy sztucznie wytworzoną tetraploidalną formą *A. plantago-aquatica* i *A. lanceolatum* mogłyby rzucić światło na rolę, jaką odgrywają w tym względzie inne procesy o znaczeniu ewolucyjnym, takie jak strukturalne zmiany chromosomów i mutacje genowe.

Analogiczne bariery seksualne natury ilościowej stwierdzone zostały i w obrębie innych rodzajów. W doświadczeniach nad krzyżowaniem diploidalnych i tetraploidalnych typów *Aquilegia* Skalińska (1937, 1945) stwierdziła tylko w rzadkich przypadkach rozwój nasion i powstawanie

organizmów triploidalnych. Organizmy te wykazały bardzo niski stopień płodności w związku z zaburzeniami w przebiegu mejozy.

Podobne zjawiska występują wg Müntzinga (1941) w obrębie rodzaju *Galeopsis*. Gatunki tetraploidalne *G. tetrahit* ($2n=32$) i *G. bifida* ($2n=32$) nie krzyżują się z gatunkami diploidalnymi *G. speciosa* ($2n=16$) i *G. pubescens* ($2n=16$) albo co najwyżej wykształcają w wyniku krzyżowania zarodki ginące we wczesnych stadiach rozwoju. Przypuszczalnie bariera seksualna pomiędzy tymi gatunkami wynika z różnic w liczbie kompleksów chromosomowych. Za słusznością tego poglądu przemawia fakt, że uzyskane eksperymentalnie autotetraploidy *G. pubescens* i *G. speciosa* nie krzyżują się z diploidalnymi formami wyjściowymi, natomiast krzyżują się stosunkowo bez trudności z tetraploidalnym gatunkiem *G. tetrahit*.

Dane taksonomiczne wskazują na odrębność gatunkową *A. plantago-aquatica* i *A. lanceolatum*, z drugiej jednak strony sugerują one istnienie związku genetycznego pomiędzy tymi gatunkami. Pozwalają one przypuszczać, że *A. lanceolatum* jest gatunkiem młodszym, w którego powstaniu brał udział gatunek *A. plantago-aquatica*.

Pewne światło na zagadnienie genezy *A. lanceolatum* rzuciły również badania kariologiczne (tab. 2). Wykazały one, że *A. plantago-aquatica* jest gatunkiem diploidalnym, reprezentowanym najczęściej przez biotypy $2n=14$. W różnych partiach zasięgu tego gatunku stwierdzono również typy o liczbach chromosomów $2n=10, 12, 16$. Natomiast *A. lanceolatum* jest gatunkiem tetraploidalnym, w obrębie którego znaleziono typy o liczbach $2n=24, 26, 28$. Liczba chromosomów $2n=24$ została podana dotychczas tylko przez Tarnavskiego (1948, wg Löve i Löve w druku) dla roślin pochodzących z Rumunii. Cytotypy $2n=28$ zostały znalezione w bardziej północnej partii zasięgu *A. lanceolatum* (Anglia, Szwecja, Finlandia, Dania), natomiast cytotypy $2n=26$ — bardziej na południe od poprzednich (Dania, okolice Wiednia, Portugalia, Francja). Tylko w Danii zatem występują obydwie te cytotypy.

W oparciu o kryteria kariologiczne, geneza *A. lanceolatum* była dyskutowana przez Erlandssona (1946) oraz przez Tschermak-Woess (1948). Erlandsson przypuszcza, że *A. lanceolatum* jest autotetraploidem powstałym w wyniku podwojenia liczby chromosomów *A. plantago-aquatica* ($2n=14$). Wręcz odmienny pogląd na zagadnienie genezy *A. lanceolatum* wyraża Tschermak-Woess. Autorka ta przypuszcza, że badane przez nią formy *A. lanceolatum* ($2n=26$) są pochodzenia allopoliploidalnego i wywodzą się od typów $2n=14$, o morfologii chromosomów zupełnie podobnej do określonej przez nią dla *A. gramineum* i *A. plantago-aquatica*, oraz typów $2n=12$, jakie według wszelkiego prawdopodobieństwa występują w obrębie *A. plantago-aquatica*.

Na poparcie swojej koncepcji autorka wzmiankuje, że pewne cechy morfologii zewnętrznej *A. lanceolatum* mają charakter pośredni pomiędzy cechami domniemanych form rodzicielskich.

Koncepcja Tschermak-Woess, dotycząca pochodzenia *A. lanceolatum*, nie zdaje się jednak mieć poparcia w danych morfologicznych; nie mogą też mieć znaczenia rozstrzygającego kryteria wyłącznie kariologiczne, gdyż, jak sama autorka podkreśla, kariotypy *A. plantago-aquatica* i *A. gramineum* są identyczne.

Jak widać z dotychczasowych badań systematycznych oraz z własnych obserwacji przeprowadzonych na (szczupłym wprawdzie) materiale zielnikowym (Banach-Pogan, nie publ.), szereg cech morfologicznych wyraźnie odróżnia obydwie gatunki: *A. plantago-aquatica* i *A. lanceolatum* od *A. gramineum*. W odróżnieniu od tych obydwu gatunków *A. gramineum* ma kłace słabiej zgrubiałe, wydłużone, cylindryczne. Liście form lądowych są podłużnie eliptyczne lub lancetowate, krótkoogonkowe, o szczycie i podstawie blaszki liściowej gwałtownie zwężającej się, zaś u form wodnych wstęgowate, równowąskie.

Pędy kwiatowe są ukośne albo łukowato wyrastające ku górze, albo pokładające się. Rozgałęziają się one już w dolnej połowie. Kwiatostan jest krótszy o mniejszej liczbie okółków, zwykle 3—4, gwałtownie zwężający się ku górze. Gałązki kwiatostanu są poziome lub odgięte ku dołowi. Kwiaty mniejsze; płatki 2—4 mm dł. są tylko 1,5 raza dłuższe niż działki. Pręciki tak długie jak załącznik albo nieco dłuższe od nich. Pylniki okrągławe lub owalne $\pm 0,3$ — $0,4$ mm dł., $0,3$ mm szer. Pomiedzy słupkami nie ma wolnej przestrzeni, gdyż załącznik stykają się ze sobą krawędziami brzuszными. Szyjki słupków, łącznie ze znamieniem krótsze niż załącznik lub co najwyżej tak długie jak załącznik, $\pm 0,3$ — $0,7$ mm dł., odgięte haczykowato lub spiralnie na zewnątrz; znamiona słupków grubobrodawkowane. Owoce mają na stronie grzbietowej dwa rowki, ograniczone trzema żeberkami ostrymi o jednakowej wysokości.

Zatem z uwagi na cechy morfologiczne i wyniki analizy cytologicznej udział *A. gramineum* w powstaniu *A. lanceolatum* jest raczej wątpliwy; wydaje się bardziej prawdopodobne, że tetraploidalny gatunek *A. lanceolatum* powstał w obrębie diploidalnego gatunku *A. plantago-aquatica* być może jeszcze z udziałem jakiegoś wygasłego gatunku rodzaju *Alisma*. Badania kariologiczne rzucają światło na domniemane szlaki ewolucyjne *A. lanceolatum*.

W toku niniejszych badań nad licznymi populacjami obydwu gatunków ze stanowisk naturalnych w Polsce jak również, dla porównania, potomstwa roślin z Ogrodu Botanicznego w Cambridge (pochodzącego z Nantes) została stwierdzona dla *A. plantago-aquatica* tylko jedna liczba chromosomów $2n=14$ oraz jedna tylko liczba chromosomów $2n=26$ dla

A. lanceolatum With. Obydwa gatunki nie wykazują na obszarze Polski zróżnicowania kariologicznego.

Liczba chromosomów *A. lanceolatum* $2n = 26$, podawana już uprzednio dla materiałów z innych krajów (Löve i Löve 1942; Tschermak-Woess 1948; de Castro i Noronha-Wagner 1950) nie jest liczbą ściśle euploidalną. Liczbowe zestawienie typów chromosomów zamieszczone w pracy Tschermak-Woess nasuwa wprawdzie pewne sugestie dotyczące powstania tego kariotypu, niemniej ani w pracy autorki, ani w innych dotychczasowych publikacjach nie spotkałam się z interpretacją jego genezy. Nie zostały również opracowane dotychczas dokładnie dla gatunków rodzaju *Alisma*, występujących na terenie Europy, idiogramy, które umożliwiłyby porównanie kariotypów. Dopiero tego rodzaju badania mogłyby wyjaśnić genezę *A. lanceolatum* z kariologicznego punktu widzenia.

Ogromna większość liczb chromosomów podawanych dla gatunków *Alisma* L. stanowi całkowitą wielokrotność liczby podstawowej $x = 7$; tę liczbę należy przeto uważać za najbardziej pierwotną u *Alisma*. W związku z tym i kariotyp $2n = 14$ powinien być uznany za najbardziej pierwotny.

Z teoretycznego punktu widzenia zatem można przypuszczać, że ewolucja kariotypu *A. lanceolatum* wyszła od ściśle diploidalnego kariotypu ($2n = 14$).

Porównanie kariotypu *A. plantago-aquatica* ($2n = 14$) z kariotypem *A. lanceolatum* ($2n = 26$) przeprowadzone w toku niniejszych badań wykazało, że powstać on mógł w wyniku co najmniej dwu procesów cytologicznych, mianowicie zmian strukturalnych chromosomów oraz poliploidności. Jak wynika z idiogramów, wyznaczonych dla obydwu gatunków (fig. 5, 6), te zmiany strukturalne dotyczyły przypuszczalnie niehomologicznych chromosomów z centromerem subterminalnym (VI, VII). Z czterech par tych chromosomów, jakie teoretycznie powinny występować w kariotypie formy euploidalnej *A. lanceolatum* ($2n = 28$), występuje tu tylko jedna para chromosomów dłuższych (VI) i jedna para chromosomów krótszych (VII). Pozostałe dwie pary są u *A. lanceolatum* zastąpione przez jedną parę chromosomów z centromerem submedialnym (VIII), których nie ma w kompleksie *A. plantago-aquatica* ($2n = 14$).

Ten nowy typ chromosomów powstał najprawdopodobniej w wyniku nierównej translokacji, jaka dokonała się pomiędzy niehomologicznymi chromosomami z centromerem subterminalnym, oraz eliminacji fragmentu centrycznego przypuszczalnie genetycznie inercyjnego.

Zmiany te mogły zajść na szczeblu diploidalnym. Ich naturalną konsekwencją były zaburzenia normalnego przebiegu procesu mejozy u odpowiednich aberantów i powstawania w coraz dalszych pokoleniach

form zdolnych do wytwarzania gamet nie zredukowanych $n=12$ oraz $n=14$, zawierających łącznie całkowity zespół chromosomów występujących u *A. lanceolatum* ($2n=26$). Oczywiście wyżej podany schemat mógł być realizowany w różnych, teoretycznie równie możliwych kombinacjach. Nie jest wykluczona również i ta możliwość, że zmiany strukturalne chromosomów zaszyły na szczeblu tetraploidalnym i dokonały się w obrębie kompleksu formy euploidalnej. Poliploidalność w takim razie byłaby etapem ewolucyjnym poprzedzającym zmiany strukturalne chromosomów. Strukturalne zmiany chromosomów mogły znaleźć odzwierciedlenie w morfologii roślin i stanowić jeden z etapów wyodrębniania się nowego gatunku. W jego różnicowaniu się mogły odegrać również rolę mutacje genów.

Na możliwość wyjaśnienia zróżnicowania kariologicznego w obrębie *A. plantago-aquatica* w wyniku strukturalnych zmian chromosomów wskazują już Löve i Löve (1942), jak również Darlington (1956).

Rozstrzygające znaczenie dla powyżej przedyskutowanych zagadnień miałyby badania kariologiczne i morfologiczne roślin z całego zasięgu tych gatunków, szczególnie z obszarów, gdzie wykazują one zróżnicowanie kariologiczne.

Występowanie większych zmian strukturalnych chromosomów, prowadzące do ewolucji kariotypu oraz zmian w podstawowych liczbach chromosomów, było stwierdzone dla szeregu grup roślinnych. Tendencja ku filogenetycznej redukcji liczb chromosomów była obserwowana zarówno w obrębie okrytozależkowych, jak u nagozależkowych i mchów.

Jednym z najwcześniej poznanych pod tym względem rodzajów był rodzaj *Fritillaria* (Darlington 1937, str. 77). Osiem gatunków tego rodzaju, uwzględnionych w zestawieniu, ma $n=12$ (2 „V” + 10 „I”). Z gatunków różniących się od nich liczbą chromosomów *F. ruthenica* ma $n=9$ (5 „V” + 4 „I”) i powstała przypuszczalnie w wyniku „fuzji”, natomiast *F. pudica* ($n=13$) (1 „V” + 12 „I”) w wyniku fragmentacji z form 12-chromosomowych.

Udział zmian strukturalnych chromosomów obok innych procesów ewolucyjnych był podkreślany również w różnicowaniu się kariotypów w obrębie rodzaju *Lycoris* (Inariyama 1932, 1937; Satô 1938, Bose 1958a, b). U *Lycoris* można wyróżnić dwie zasadnicze klasy chromosomów: chromosomy „V” z centromerem medialnym, lub prawie medialnym, oraz chromosomy „I” z centromerem niemal terminalnym. Zbadane kariologicznie gatunki rodzaju *Lycoris* (Bose 1958b — tabela 4) różnią się między sobą liczbami chromosomów oraz liczbą występujących w ich kariotypach chromosomów „V”, oraz „I”. Niektóre mają wyłącznie chromosomy „I” (*L. haywardii* — $2n=22$, *L. radiata* var. *pumila* — $2n=22$, *L. radiata* — $2n=33$, *L. sanguinea* $2n=22$, *L. sprengeri* —

$2n = 22$). U innych natomiast występują zarówno chromosomy „I”, jak i chromosomy „V”, przy czym istnieje regularna liczbową zależność pomiędzy tymi dwiema klasami chromosomów. Jeśli przyjmujemy — miałowicie — że chromosom „V” jest odpowiednikiem dwu chromosomów „I”, wówczas wszystkie liczby chromosomów występujące u *Lycoris* stanowią całkowitą wielokrotność liczby $x = 11$ (przy założeniu, że są to tylko chromosomy „I”). Wydaje się więc prawdopodobne, że ewolucja kariotypu u *Lycoris* odbywała się w wyniku zmian strukturalnych chromosomów, polegających na „fuzji” chromosomów „I” (obustronnej translokacji połączonej z eliminacją fragmentu centrycznego) albo fragmentacji chromosomów „V” (zaburzeń w podziale centromeru). Wskazuje na to występowanie triwalentów u niektórych gatunków rodzaju *Lycoris* (Inariyama 1932, 1937). Dziś nie jest już możliwe stwierdzenie, który z powyższych dwu procesów — fuzja czy fragmentacja — miał znaczenie decydujące w ewolucji kariotypu *Lycoris*. Inariyama (1937) wychodząc z założenia, że u *Amaryllidaceae* najczęściej występuje liczba podstawowa $x = 11$ przypuszcza, że najbardziej pierwotny jest kariotyp $2n = 22$ zbudowany z chromosomów „I”, występujący u *L. sanguinea*. Inne kariotypy powstały z niego w wyniku fuzji chromosomów „I” oraz innych procesów ewolucyjnych, takich jak poliploidalność i krzyżowanie. Natomiast Darlington (1956) jak również Bose (1958a) są raczej skłonni wyjaśniać strukturalne zmiany chromosomów u *Lycoris* fragmentacją chromosomów „V” (zaburzeniami w podziale centromeru i powstawanie tzw. izochromosomów).

Również zjawiskiem fuzji chromosomów tłumaczy Satō (1937, 1938) ewolucję kariotypu w obrębie rodzaju *Leucojum*. *L. vernum*, *L. vernum* var. *carrathicum* i *L. aestivum* mają w płytkach somatycznych 22 chromosomy (jedna para chromosomów „V” z centromerem medialnym, zaś pozostałe, różnej wielkości, z centromerem subterminalnym). *L. autumnale* ma natomiast somatyczną liczbę $2n = 14$ (pięć par chromosomów „V” oraz dwie pary chromosomów „I”). Satō uważa, że kariotyp *L. autumnale* $2n = 14$ powstał z kariotypu $2n = 22$ w wyniku fuzji czterech par chromosomów „I”.

Fuzja chromosomów odegrała również przypuszczalnie rolę (obok innych procesów ewolucyjnych) w różnicowaniu kariotypu w obrębie rodzaju *Scilla* (Satō 1935, 1936).

Zagadnienie ewolucji kariotypu zostało opracowane w sposób najbardziej wszechstronny dla rodzaju *Crepis* (Babcock 1947a, b; Stebbins 1951). Różnicowanie się kariotypu w obrębie rodzaju *Crepis* odbywało się w wyniku skomplikowanych procesów ewolucyjnych takich, jak zmiany strukturalne chromosomów, wzrost asymetrii chromosomów, redukcja ich całkowitej długości, jak również poliploidalność i krzyżowanie. Najbardziej wyraźną cechą ewolucji kariotypu u *Crepis* jest

progresywnie obniżenie podstawowej liczby chromosomów. Mechanizm tego procesu został zanalizowany przez Tobgy'ego (1943) na podstawie badań cytogenetycznych przeprowadzonych na blisko ze sobą spokrewnionych gatunkach rodzaju *Crepis*: *C. neglecta* ($n = 4$) i *C. fuliginosa* ($n = 3$) jak również ich mieszańców. Z porównania idiogramów tych dwu gatunków oraz sposobu koniugacji chromosomów u mieszańców autor wyciąga wniosek, że kariotyp *C. fuliginosa* powstał przypuszczalnie z kariotypu *C. neglecta* (albo z kariotypu wygasłych już przodków tego gatunku) w wyniku obustronnych translokacji pomiędzy niehomologicznymi chromosomami. W kompleksie chromosomowym *C. fuliginosa* brak jednego chromosomu występującego u *C. neglecta*; jeden fragment tego chromosomu — acentryczny — uległ zespoleniu z jednym z chromosomów *C. fuliginosa*, natomiast drugi fragment, centryczny, przypuszczalnie genetycznie inercyjny, uległ eliminacji.

Jak wynika z badań Haira i Beuzenberga (1958) przeprowadzonych na 52 gatunkach z rodziny *Podocarpaceae*, również u roślin nagonasiennych wyraźnie zaznacza się rola strukturalnych zmian chromosomów w ewolucji kariotypu. U *Podocarpaceae*, przy każdej sukcesywnej zmianie liczby podstawowej, chromosom „V” zastępuje („fuzja”) albo jest zastępowany („fragmentacja”) przez dwa chromosomy „I”. Potwierdzeniem tych przypuszczeń jest występowanie w mejozie u naturalnego mieszańca ($2n = 36$) pomiędzy blisko spokrewnionymi gatunkami *Podocarpus nivalis* ($2n = 38$) i *Podocarpus hallii* ($2n = 34$) 15 biwalentów oraz 2 triwalentów. Obserwacje nad budową kariotypu dzikiej populacji *Podocarpus longifolius* ($2n = 38, 37$) oraz hodowanej populacji *Podocarpus elatus* ($2n = 36, 35$) wskazują na to, że nie fragmentacja chromosomów, ale raczej ich „fuzja” (nierówna wymiana segmentów niehomologicznych chromosomów „I” połączona z eliminacją fragmentu centrycznego, przypuszczalnie inercyjnego) odegrała rolę w różnicowaniu kariotypu u *Podocarpaceae*. Następstwem jej było progresywne obniżanie liczby podstawowej chromosomów. Proces ten rozpoczął się przypuszczalnie od liczby podstawowej $x = 20$ (chromosomy „I”) dając następnie serię liczb podstawowych $x = 19, 18, 17, 15, 13, 12, 11, 10, 9$ występujących u *Podocarpaceae*.

Zjawiskiem fuzji chromosomów tłumaczy Vaarama (1950, 1953, 1954) powstanie kariotypu $n = 5$ występującego u *Pleurozium schreberi* (= *Hylocomium Schreberi*). Dwa spokrewnione z *P. Schreberi* gatunki: *Ptilium crista castrensis* i *Hylocomium splendens* mają haploidalną liczbę chromosomów $n = 11$. Jeden z tych chromosomów jest heterochromatynowy, natomiast 10 pozostałych — euchromatynowe. Według autora haploidalny kompleks chromosomowy *P. schreberi* — $n = 5$ mógł powstać z kompleksu $n = 11$ w wyniku fuzji całych chromosomów („end-tu-end

fusion“). Największy chromosom kompleksu chromosomowego *P. schreberi* („A“) stanowi produkt fuzji trzech chromosomów: dwu euchromatynowych oraz trzeciego chromosomu heterochromatynowego.

W powyżej omówionych przykładach, podobnie jak u *Alisma lanceolatum*, zmiany strukturalne chromosomów prowadzą do zmian w liczbie chromosomów.

Zróżnicowanie kariologiczne *A. lanceolatum* zdaje się wykazywać związek z rozmieszczeniem geograficznym, a typy kariologiczne $2n = 26$ i $2n = 28$ mają przypuszczalnie charakter ras geograficznych. Cytotypy $2n = 28$ przestawiałyby rasę występującą w najbardziej północnej partii zasięgu *A. lanceolatum*, natomiast zasięg cytotypu $2n = 26$ rozciąga się bardziej na południe. Wykrycie w materiale z Polski wyłącznie biotypów $2n = 26$ przemawia na korzyść powyższej koncepcji.

Być może że wyrazem tego zróżnicowania jest, obok liczby chromosomów, różnica w długości szyjek słupków określonych przez Samuelsona (1932) dla materiałów z całego zasięgu *A. lanceolatum* oraz ustalonych w toku niniejszych badań dla biotypów $2n = 26$.

STRESZCZENIE WYNIKÓW

Zagadnieniem niniejszej pracy było ustalenie wzajemnego stosunku dwu gatunków rodzaju *Alisma* L., mianowicie *A. plantago-aquatica* L. i *A. lanceolatum* With. oraz próba wyjaśnienia genezy *A. lanceolatum* ($2n = 26$).

Zagadnienia te zostały rozważone w oparciu o połączone badania cytologiczne i morfologiczne obejmujące liczne populacje obydwu gatunków.

Zbadany kariologicznie materiał *A. plantago-aquatica* pochodził z 64 stanowisk naturalnych na obszarze całej Polski; materiał znacznie rzadszego we florze Polski gatunku *A. lanceolatum* był szczuplejszy; pochodził on z 10 stanowisk naturalnych w różnych okolicach Polski.

Dla porównania został też opracowany materiał obydwu gatunków otrzymany z Ogrodu Botanicznego w Cambridge (pochodzenie materiału: Nantes, Francja).

W toku badań kariologicznych zostały ustalone następujące liczby chromosomów:

A. plantago-aquatica L. — $2n = 14$.

A. lanceolatum With. — $2n = 26$.

Wyniki badań kariologicznych, jak również morfologicznych i anatomicznych, wskazują na odrębność gatunkową *A. lanceolatum*, podkreślając przy tym genetyczny związek tego gatunku z *A. plantago-aquatica* L.

Porównanie morfologii chromosomów obydwu gatunków pozwala przypuszczać, że kariotyp *A. lanceolatum* $2n = 26$ wykształcił się w wyniku

zmian strukturalnych chromosomów oraz poliploidalności z kariotypu $2n = 14$ występującego u *A. plantago-aquatica*.

Zmiany strukturalne chromosomów *A. lanceolatum* można interpretować jako wynik obustronnej translokacji oraz eliminacji fragmentu centrycznego, przypuszczalnie genetycznie inercyjnego. Zmiany ewolucyjne kariotypu, mutacje genowe i być może krzyżowanie były przypuszczalnie czynnikami, które doprowadziły do wyodrębnienia się nowego gatunku *A. lanceolatum*.

Wśród zbadanych okazów zostały wykryte trzy rośliny mające w tkankach somatycznych po 20 chromosomów. Jak wykazała analiza ich kariotypów oraz obserwacje morfologiczne i anatomiczne, są to mieszańce *A. plantago-aquatica* $\times$ *A. lanceolatum*. Występowanie mieszańców pomiędzy tymi gatunkami nie było dotychczas notowane w literaturze botanicznej.

Na terenie Polski i we Francji występuje typ kariologiczny *A. lanceolatum* — $2n = 26$ podobnie jak w Danii, Austrii i Portugalii. Natomiast drugi typ — $2n = 28$ podawany był z najbardziej północnej części zasięgu tego gatunku.

Badania przeprowadzone na materiale z Polski przemawiają na korzyść przypuszczenia, że gatunek ten wykazuje zróżnicowanie na dwie rasy geograficzne, odpowiadające dwu typom kariologicznym — bardziej północną — $2n = 28$, oraz na południe od niej występującą rasę — $2n = 26$.

Praca została wykonana z częściowym zasiłkiem Komitetu Botanicznego PAN

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(Wpłynęło: 26.6.1961 r.)

SUMMARY

The purpose of the present study was to establish the mutual relation of *Alisma plantago-aquatica* L. and *A. lanceolatum* With., as well as to elucidate the problem of origin of *A. lanceolatum* from the karyological point of view.

According to the opinion of taxonomists these two species are very closely related. However, in view of morphological and phytogeographical differences occurring between them they are considered by recent taxonomists as separate species.

The present morphological investigations carried out on the Polish material confirm and supplement these results. In addition to the morphological features previously taken into consideration (shape of leaf blades, shape of petals, size of the stigma papillae), they led to establish some anatomical differences (length of stomata, Fig. 9, diameter of pollen grains, Fig. 11) which are helpful for distinguishing these two species.

Special attention has been paid to the length of styles — a feature of a high diagnostic value. According to Samuelsson (1932) the length of the styles of *A. plantago-aquatica* ranges from 1 to 1¼ mm.; that of *A. lanceolatum* from ¾ to 1½ mm. The resp. data established for the Polish material are: (0.7) 0.8 to 1.43 mm. for *A. plantago-aquatica* and (0.3) 0.4 to 0.83 mm. for *A. lanceolatum* (Figs 12, 17—25, 28—32). Thus, in Poland the latter species has notably shorter styles. The above data are in accordance with the results of Glück (1936) based on materials from Central Europe.

In view of the occurring differences *A. lanceolatum* is regarded as a separate species; it may be assumed that *A. plantago-aquatica* has taken part in its origin.

Karyological investigations done hitherto (Table 1) throw some new light on the problem of origin of *A. lanceolatum*. They showed that *A. plantago-aquatica* is a diploid species and its most frequent cytotype is $2n = 14$. In some parts of the area of its distribution *A. plantago-aquatica* shows some degree of karyological differentiation: the cytotypes $2n = 10, 12, 16$ could be found there in addition to the largely distributed cytotype $2n = 14$; they are, however, notably rarer than the latter. On the other hand, *A. lanceolatum* is a tetraploid species. It is differentiated into three cytotypes with $2n = 24, 26, 28$ resp. The number $2n = 24$ was established till now only by Tarnavský (1948, cit. from Löve and Löve, in press) for specimens originating from Rumania. The cytotypes $2n = 28$ have been found in the northern part of the area of distribution of *A. lanceolatum* (Great Britain, Sweden, Finland, Denmark); by contrast, the occurrence of cytotypes $2n = 26$ was established more southwards (Denmark, vicinity of Vienna, Portugal, as well as France: Nantes, established in the course of the present study). Thus, a simultaneous occurrence of these two cytotypes has been established till now only in Denmark.

The problem of origin of *A. lanceolatum* was discussed from the karyological point of view by Erlandsson (1946) and Tschermak-Woess (1948). According to Erlandsson, the slight morphological differences between these two species, as well as their chromosome numbers, indicate that *A. lanceolatum* ($2n = 28$) is an autotetraploid derived from *A. plantago-aquatica* ($2n = 14$) in the way of doubling of the chromosome number.

By contrast, Tschermak-Woess assumed that the forms of *A. lanceolatum* ($2n = 26$) studied by her are allopolyploids which originated from diploid types with $2n = 14$, possessing karyotypes quite similar to those found by this author in *A. gramineum* and *A. plantago-aquatica*, and from the types $2n = 12$ which seem to occur among the forms of *A. plantago-aquatica*. This author emphasized that some morphological features of *A. lanceolatum* are apparently intermediate between those of the putative parental forms.

The results of the morphological observations carried out on the Polish material do not favour this assumption. They are rather in accordance with the opinion suggesting the origin of *A. lanceolatum* from some forms of *A. plantago-aquatica*, possibly with the part of some extinct species within the genus.

Some points of the problem of origin of *A. lanceolatum* could be elucidated in the course of the present study. *A. plantago-aquatica* is very common in the Polish flora. Numerous strains of this species were studied in the course of the present work. They originated from 64 natural habitats distributed over the whole area, extending from the Baltic coast to the foreland of the Tatra Mts. In ten of these habitats specimens of *A. lanceolatum* which is a rather rare species in our flora, occurred together with *A. plantago-aquatica* (Table II); specimens of these two species grew there in close neighbourhood, not infrequently in the same tufts.

In our flora the two species seem to be karyologically uniform. Only one chromosome number was invariably found for each of them in all specimens studied, namely $2n = 14$ for *A. plantago-aquatica* and $2n = 26$ for *A. lanceolatum*.

The list of chromosome numbers given for the species of *Alisma* (Table 1) permits to infer that the basic number in this genus is $x = 7$. Therefore within the genus the cytotype with $2n = 14$ has the most primitive karyotype, corresponding with that occurring in *A. plantago-aquatica*. The karyotype of *A. lanceolatum* with $2n = 26$ may have evolved in consequence of at least two different cytological processes: the doubling of the chromosome number, as well as some structural changes leading to a decrease of the chromosome number.

The comparison of the idiograms of *A. plantago-aquatica* ($2n = 14$) and *A. lanceolatum* ($2n = 26$) (Figs. 5, 6) suggests that these structural changes concerned two nonhomologous chromosomes with subterminal centromeres (VI, VII). Of two pairs of the longer (VI) and two pairs of the shorter chromosomes (VII) which ought to be present in the karyotype of an euploid form of *A. lanceolatum* ($2n = 28$), only one pair of each type of these chromosomes occurs in the form $2n = 26$. The two remaining pairs are replaced in this form by one pair of chromosomes with a submedian centromere (VIII), a type which is absent from the karyotype of *A. plantago-aquatica* ($2n = 14$). This new type of chromosomes could have arisen through the process of reciprocal translocation between the nonhomologous chromosomes with subterminal constriction, connected with the elimination of the centric fragment which is probably genetically inert.

Thus, the present karyological investigation brings new evidence in favour of the distinctness of *A. lanceolatum* as a separate species and throws light on the way in which it could have arisen.

In the ample material originating from different habitats in Poland three plants with 20 somatic chromosomes were found. The analysis of their karyotype (Fig. 7) as well as preliminary morphological and anatomical observations led to identify the resp. plants as hybrids between *A. plantago-aquatica* and *A. lanceolatum*. It should be added that hybrids between these two species were not reported hitherto.

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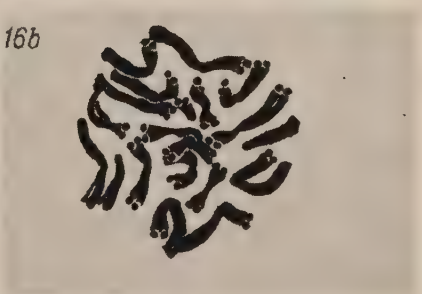
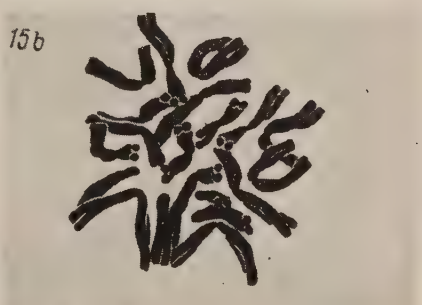
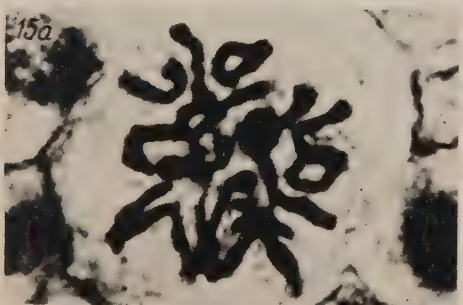
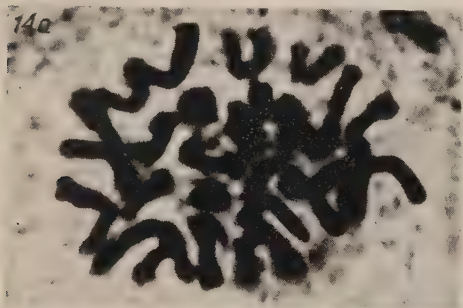
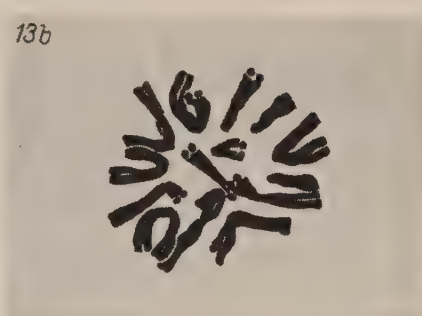
Explanations of the Plates I—XI; figs 13—44

Objaśnienia do tablic I—XI; fig. 13—44

- Fig. 13—16. Mikrofotografie oraz odpowiednie rysunki płytek metafazowych w stożkach wzrostu korzeni (pow. mikrofotografii 2000 ×, rysunków — c. 2000 ×)
Microphotos and the corresponding drawings of the root-tip metaphases (microphotos — 2000 ×, drawings — c. 2000 ×)
13a, 13b — *Alisma plantago-aquatica* L. (2n = 14); 14a, 14b — *A. lanceolatum* With. (2n = 26); 15a, 15b, 16a, 16b — *A. plantago-aquatica* L. × *A. lanceolatum* With. (2n = 20)
- Fig. 17—32. Fotografie słupków (materiał alkoholowy z roślin zbadanych karyologicznie; w nawiasach — numery populacji, ob. Tabela 2)
Photos of the pistils (alcohol material from specimens investigated karyologically; in brackets — the corresponding strain N., see Tabl. 2)
- Fig. 17—25. *Alisma plantago-aquatica* L. (2n = 14); 17 — (Nr 101); 18 — (Nr 123a); 19 — (Nr 129); 20 — (Nr 124); 21 — (Nr 127); 22 — (Nr 122c); 23 — (Nr 108); 24 — (Nr 8); 25 — (Nr 142)
- Fig. 26—27. *Alisma plantago-aquatica* L. × *A. lanceolatum* With. (2n = 20); 26 — (Nr 105); 27 — (Nr 123b)
- Fig. 28—32. *Alisma lanceolatum* With. (2n = 26); 28 — (Nr 122b); 29 — (Nr 122a); 30 — (Nr 12); 31 — (Nr 55); 32 — (Nr 126)
- Fig. 33—44. Fotografie roślin (odstęp podziałki — 10 cm) oraz należących do nich kwiatów (wielkość naturalna); w nawiasach numery populacji
Photos of herbarium specimens (intervals of the scale — 10 cm), and their flowers (nat. size); in brackets strain N.
- Fig. 33—38. *Alisma plantago-aquatica* (2n = 14); 33 — (Nr 123a); 34 — (Nr 122b); 35 — (Nr 76); 36 — (Nr 129); 37 — (Nr 101); 38 — (Nr 124)
- Fig. 39—41, 43. *Alisma lanceolatum* With. (2n = 26); 39 — (Nr 122d); 40 — (Nr 122a); 41 — (Nr 55); 43 — (Nr 140)
- Fig. 42, 44. *A. plantago-aquatica* L. × *A. lanceolatum* With. (2n = 20); 42 — (Nr 105); 44 — (Nr 123b)

Fot. J. Rychlewski

Tablica I — Plate I



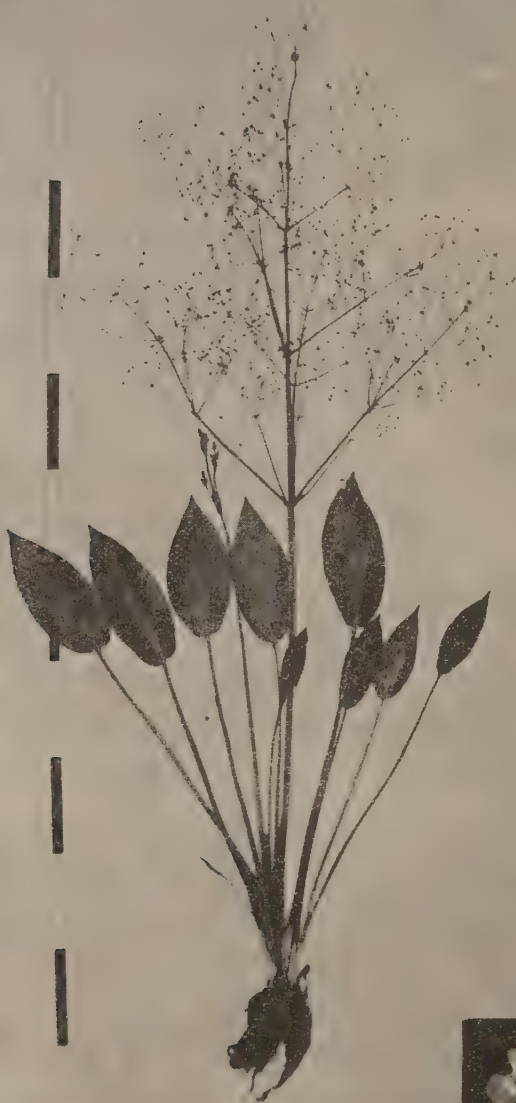
Tablica II — Plate II



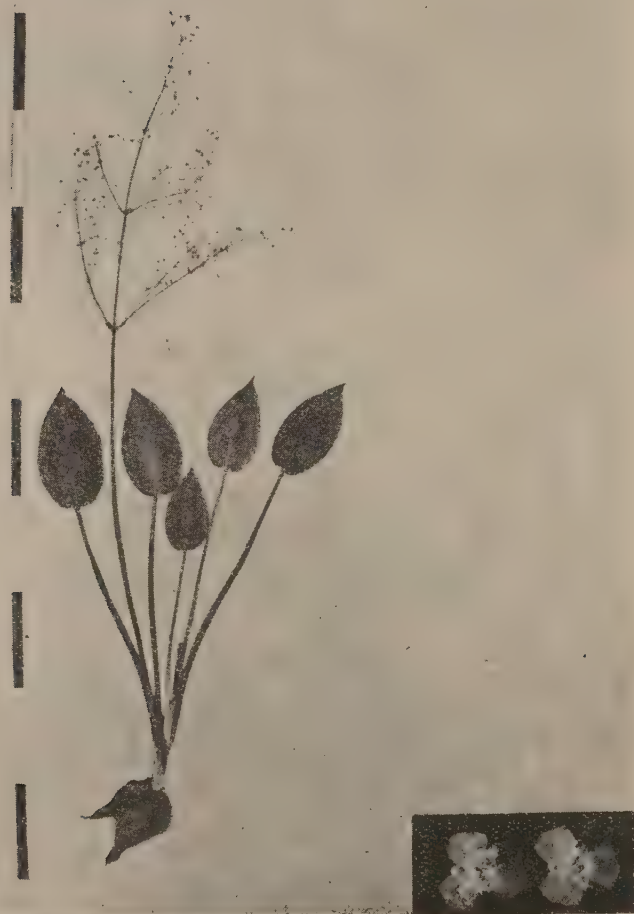


Tablica IV — Plate IV

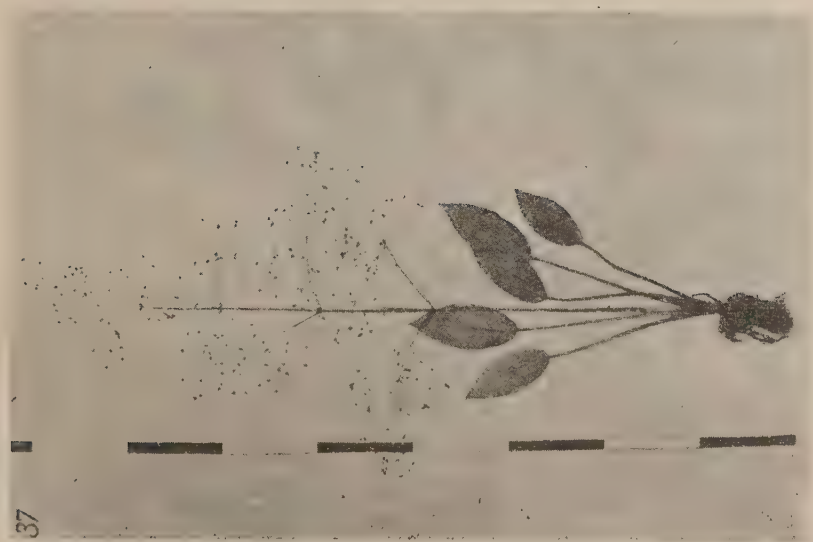
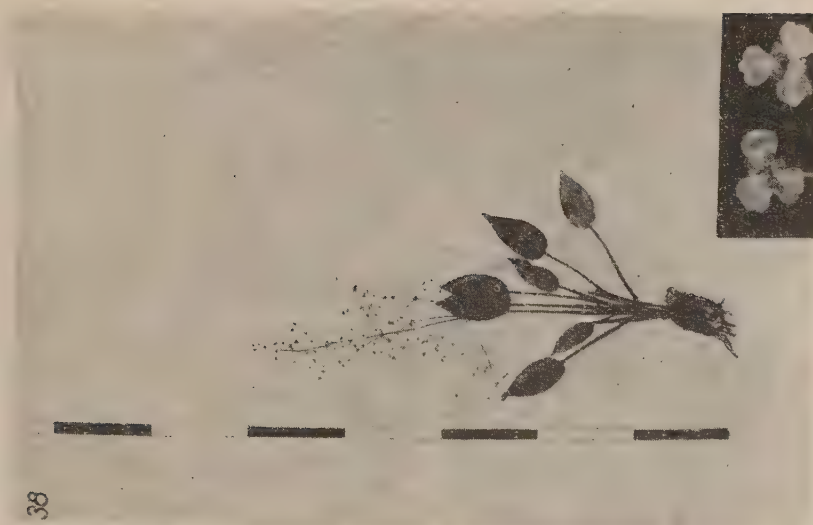
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Tablica VII — Plate VII



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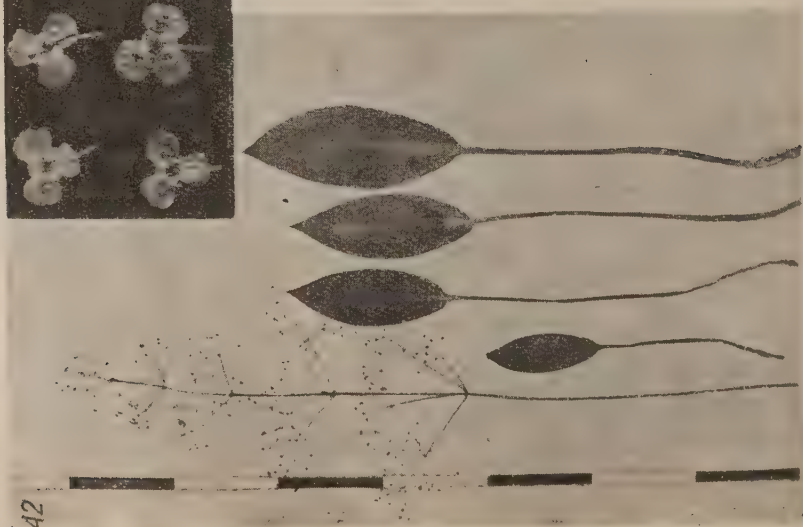
Tablica IX — Plate IX



Tablica X — Plate X



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Tablica XI — Plate XI

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Anticlinal divisions, intrusive growth, and loss of fusiform initials in nonstoried cambium*

Z. HEJNOWICZ

The laying down of wood increases the distance of the cambium from the pith and, thus, the girth of the cambium must also increase. In the case of nonstoried cambium the decisive part in this process is played by intrusive growth of fusiform cambial cells, which consists in that these cells grow at the tips forcing their way inbetween the radial walls of the neighbouring cells and push them apart (Klinken 1914, Bailey 1923, Whalley 1950, Bannan 1956). Owing to anticlinal divisions the increase of the cambium girth is accompanied by an increase in the number of fusiform initials: an initial divides into two shorter ones lying one above the other (Bailey 1923). According to Klinken these divisions are transverse and take place midway along the length of an initial: the oblique position of the cell-wall is caused, in the opinion of that author, by secondary intrusive growth of the newly formed tips of these cells. Contrary to this opinion Bailey believes that the division wall assumes from the start an oblique position. Whalley (1950) and Bannan (1957) share the opinion that the divisions are of the pseudotransverse and not the transverse type reported by Klinken.

The anticlinal divisions increase the number of fusiform initials in the nonstoried cambium and at the same time they constitute the mechanism counteracting the average-length growth of these cells caused by intrusive growth. They also directly influence the rate of intrusive growth. According to Bannan (1956) and Whalley (1950) the most rapid intrusive growth takes place immediately after an anticlinal division.

Klinken (1914), Whalley (1950), Bannan (1951, 1953), Barghorn (1940, 1941), and Evert (1960) report that the nonstoried cambium is permanently losing some of the fusiform initials in the course of its development. According to Klinken this happens because in the cambium zone the cambial cells with a greater turgor force themselves sideways inbetween the tangential walls of cells in the radial

* This report was awarded the prize in the competition for researches in developmental anatomy announced in 1960 by the Committee of Botany of the Polish Academy of Sciences.

row, where the loss of the initial is to take place, causing breaks in this row.

Whalley (1950) as well as Bannan and Bayly (1956) state that just before an initial cell loses its initial function it is shortened and its tangential width decreases. Simultaneously the cell often goes through several anticlinal divisions and in the course of this process all or some of the segments may be eliminated. The remaining segments give rise to new rays (Bannan 1956). The elimination rate of cells in the cambium of older stems with a very slight thickness growth is sufficiently high to prevent the number of these cells from increasing, in spite of the constantly proceeding anticlinal divisions of fusiform initials. E.g., among eleven fusiform initials in the cambium of *Thuja* twelve anticlinal divisions occurred during a certain period, but only ten initials remained, though, if they all were to survive there would have been at the end of that period 23 of them (Bannan 1960).

Bannan and Bayly (1956) have demonstrated that there is a tendency to eliminate mainly the shorter initials. They have also found a relationship between the number of contacts of a fusiform initial with rays and the probability of its survival.

It is worth noting, moreover, that the elimination of one initial cell stimulates the specially strong intrusive growth of the neighbouring cells (Bannan 1956).

All these data on the changes occurring in nonstoried cambium have been obtained from investigations on the xylem or the phloem but not from direct examinations of the cambium itself. It seems plausible to suppose, therefore, that the picture of the development of the cambium obtained in this way is not complete. More detailed information about the changes taking place in the cambium in the course of its development can be assembled only by direct anatomical examinations. The preliminary results from such an investigation on the cambium of *Larix europea* are reported in this paper.

MATERIAL AND METHODS

The different kinds of cambium of *Larix europea* collected for examination in the first half of July were as follows:

- 1) cambium from a root with six annual growth rings,
- 2) cambium from the stem of a tree six years old taken off the surface of the 4-th ring at the height of 1.5 m.,
- 3) cambium from a tree 40 years old taken at the height of 1.2 m., and
- 4) cambium from a tree 80 years old taken at the height of 1.5 m.

The Preparation of Slides

Pieces of the surface tissue reaching into the wood having been cut out from the stems or the root the surface tissue was removed from them down to the young phloem layer. The pieces were then divided into strips about 0.8 cm. wide and the strips were cut obliquely at the lower end to mark the basal direction. Next the surface layers about 0.8 mm. thick containing the cambium inbetween the young phloem and the xylem layers were cut off the strips with a sharp razor. In this way rectangular trapeziums about 2 cm. long containing the cambium were obtained. These were immediately fixed with Cr-A-F solution (0.4—3—10) and then embedded in paraffin after dehydration in an alcohol-benzene series. Transverse sections 16μ thick of cambium from stems and 20μ thick of cambium from roots were cut on a rotating microtome taking care to obtain continuous series extending over at least one centimetre of the cambium length. The cutting was started from the top end. The sections were arranged on slides, about 60 sections in three rows on every slide. First safranin (2% solution in 50% alcohol during 5 hours) and then anilin blue (1% solution in 96% alcohol during 1 min.) was used for staining. The sections in every series were numbered marking every tenth section with an ink dot and writing the number of the first section in every row. Several series suitable for examination were obtained from every kind of cambium.

Negative photographs were obtained using a microscope fitted with a strong source of light and a projection equipment for projecting the image of the suitable part of a section onto photographic paper. Every second or fourth section was photographed and the series of negative photographs thus obtained were glued on a paper tape, which greatly facilitated the anatomical analysis. Every series consisted of about 400 photographs.

From the series of the negative photographs of transverse sections it was possible to reconstruct the spatial arrangement of cells. In particular it was possible to reconstruct the tangential sections through the cambium and to plot diagrams of these sections. The drawings thus obtained were the starting point for the analyses of the changes occurring during the cambium growth.

RESULTS

Anticlinal divisions

Fusiform initials dividing anticlinally just at the moment of fixation are seldom found in the cambium. On the other hand, there are many such fusiform initials in which anticlinal divisions occurred in the

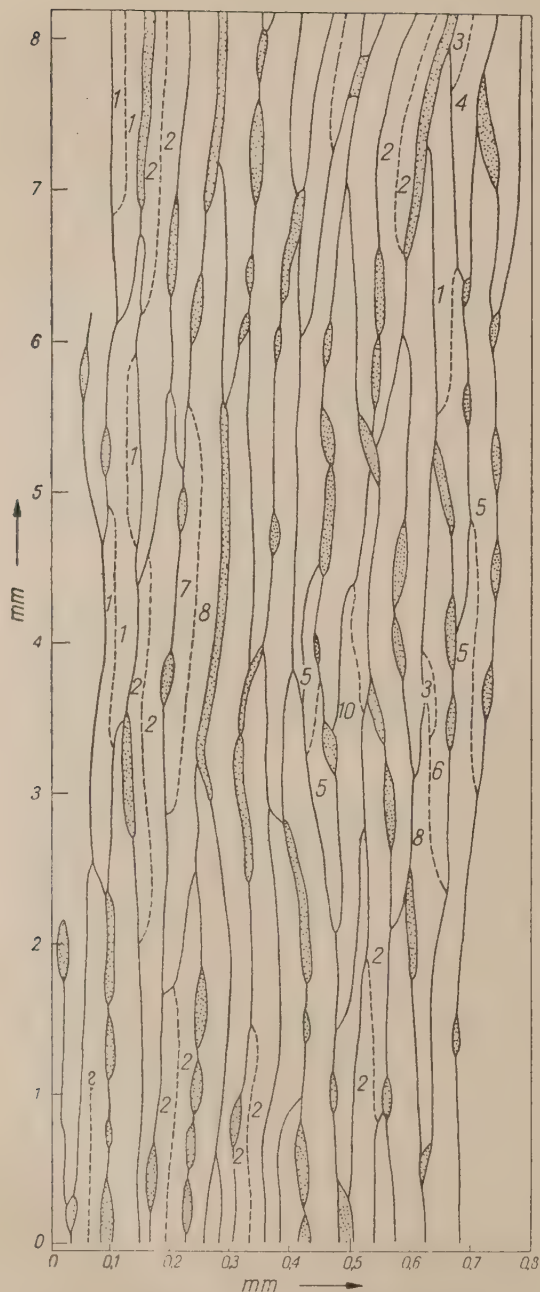


Fig. 1. Reconstructed tangential section through the root cambium as seen from the side of the phloem. The long lines correspond to the long walls of the fusiform initials and the broken lines to walls derived from anticlinal divisions in fusiform initials. The dotted areas mark the groups of the initial cells of rays. The numbers marking the anticlinal walls indicate the number of periclinal divisions, which occurred after the anticlinal divisions till the time of fixation + 1 (i.e. the number of cells produced by the new initial). The basal border of the cambium at the bottom of the figure

course of only a few periclinal divisions preceding fixation. In the cambium of the young root such cells made up about 50 per cent of all the fusiform initials, whereas the corresponding proportions in the cambium of stems were about 25 per cent for the four-year old tree and a few per

cent for trees 40 and 80 years old. (These figures do not include the divisions at the ends of the fusiform initials associated with the formation of rays, since these divisions were not considered in the course of this research).

Figures 1, 2, and 3 show the reconstructed tangential sections of the various kinds of cambium. In the diagrams the broken lines mark the

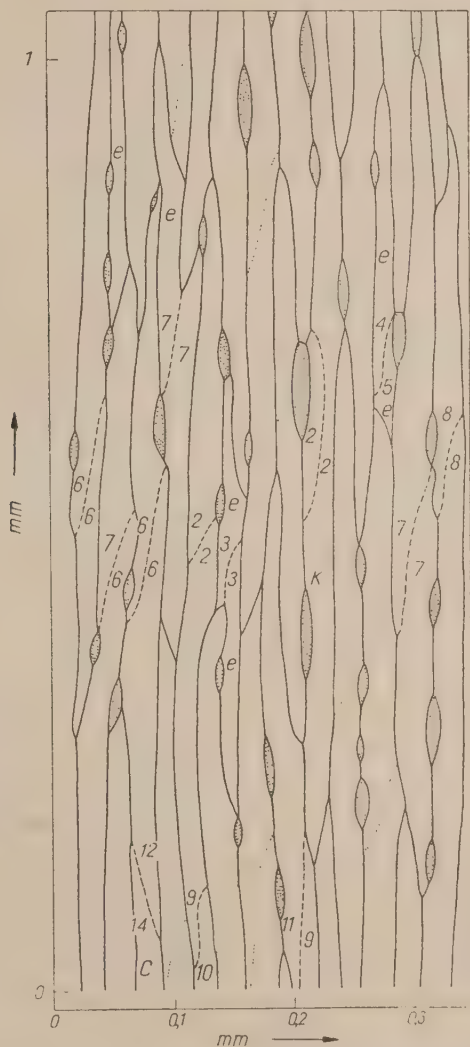


Fig. 2. The same as in fig. 1, but for the cambium of a four-year old stem. The dotted lines mark the projections of the walls from anticlinal divisions in the phloem mother cells. The initials in the course of elimination are marked with the letter "e". The one-directional character of the anticlinal divisions is well visible

walls from anticlinal divisions the number of the subsequent periclinal divisions being recorded in each case.

Let us consider first the anticlinal divisions in the cambium of the root. As is to be seen in fig. 1 the walls derived from anticlinal divisions in the fusiform initials have an appreciable length and are more or less

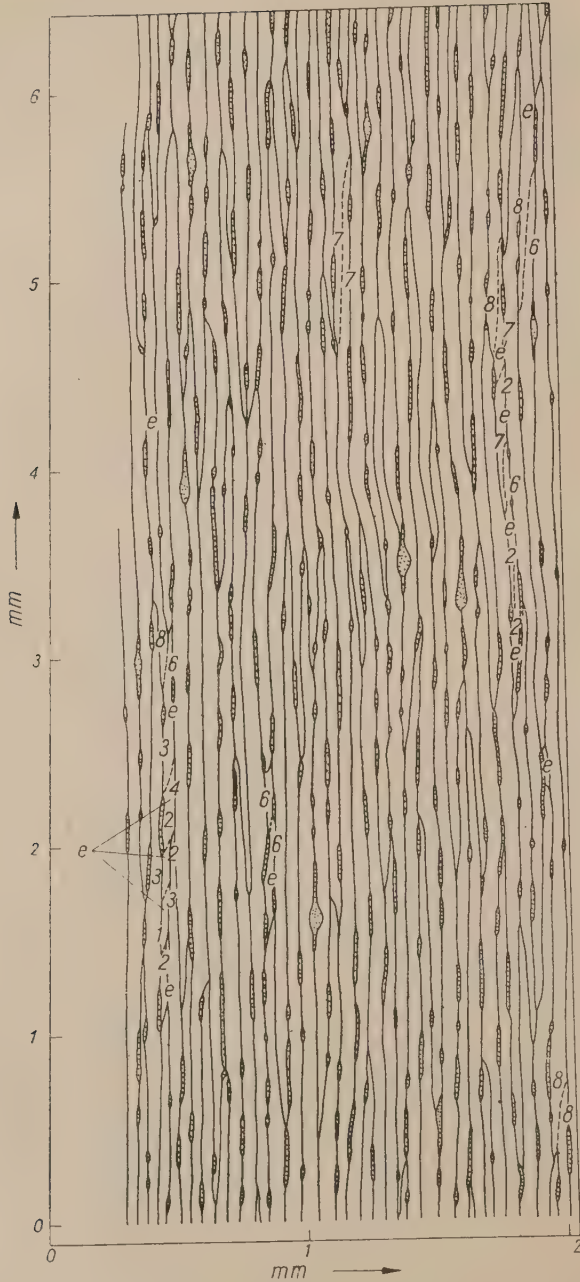


Fig. 3. The same as in fig. 1 and 2, but for the cambium of an 80-year old stem. Two kinds of anticlinal divisions are visible: 1) long divisions taking place singly in the fusiform initials, and 2) shorter ones taking place in groups in the fusiform initials undergoing elimination

symmetrically distributed with regard to the ends of the mother cells. The walls are of two kinds: either they are oblique, and then they run from one wall to the opposite wall of the mother cell, or they begin and end at the same wall of the mother cell. In the former case they result from anticlinal divisions shortening the cells, i.e. from pseudotransverse anticlinal divisions, and in the latter they result from divisions causing the separation of the long side segment.

The question now arises whether the sharply oblique position of the anticlinal walls separating the sister fusiform initials is of primary origin, i.e. produced by an oblique division, or whether its origin is secondary. In the latter instance the division would be transverse and would be followed immediately by strong intrusive growth causing the oblique arrangement of the wall separating the sister cells.

A detailed analysis of the cells derived from anticlinal divisions shows that it is possible to distinguish between the segments of walls formed in the course of cell divisions and the walls formed by intrusive growth. The part of a cell formed as the result of intrusive growth is forced inbetween neighbouring cells and owing to this it has a different appearance than the part along the plane of anticlinal division.

The series of photographs "A" in Plate 1 show fragments of root cambium containing cells from an anticlinal division: in row "a" an anticlinal division, which occurred in a fusiform initial and was followed by one periclinal division, is distinctly visible beginning with the level of 200μ . At the levels from 20μ to 160μ the upper end of a cell derived from an anticlinal division and undoubtedly formed as the result of intrusive growth already after the anticlinal division is to be seen forced inbetween the rows of neighbouring cells (in circle).

The part forced inbetween the neighbouring cells is short as compared to the part limited by the wall derived from the anticlinal division. On the photographs of the same series in row "b" there are two sister fusiform initials produced in the course of a pseudotransverse division which had not yet time to divide periclinally. (In the same row there is among the mother cells of the phloem another earlier anticlinal division which should not be mistaken for the one just mentioned). The lower end of the left initial in row "b" is visible on the last photograph of the series. There is no evidence to indicate intrusive growth in this initial derived from an anticlinal division. All this leads to the conclusion that the anticlinal divisions are not followed by intrusive growth of special intensity and, therefore, the oblique arrangement of cell-walls derived from an anticlinal division in the root cambium is of primary origin.

Mention has been made earlier that the anticlinal divisions in the root cambium can be of two kinds, i.e. there can be pseudotransverse and side

divisions. Divisions of both kinds may lead to the formation either of the fusiform initials or of cells which in the course of transverse divisions develop into ray initials.

This investigation was mainly concerned with the direction of the walls derived from pseudotransverse divisions. In the cambium of the root this direction may vary being either right- or left-handed.

In the cambium of stems there are, similarly as in the root cambium, pseudotransverse divisions, but the essential difference between the two kinds of cambium is that the pseudotransverse divisions in the cambium of stems almost exclusively follow the left-hand rotation i.e. the direction of these divisions is conformable with the direction of a left-hand spiral (it was proved that the methods of drawing the figures does not invert the direction). Among the 36 identified pseudotransverse divisions in the four-year old cambium there were only 3 with a right-hand rotation. Fig. 9 shows nine anticlinal divisions in fusiform initials of which seven have the left-hand rotation. There is one right-hand division in cell "c" and one side division in cell "k". In the four-year old cambium there are rather frequent anticlinal divisions in the phloem mother cells: the projections of the walls from these divisions onto the surface of the cambium are shown in fig. 2 by dotted lines. These divisions have also the left-hand rotation.

In the cambium of the two older stems the left-hand rotation of the pseudotransverse divisions is even more distinct. Out of the 56 examined divisions, some of which are shown in fig. 3, as many as 54 have the left-hand rotation and only two have the right-hand rotation.

Side anticlinal divisions in the fusiform initials of the cambium of stems are rare. They have been found occasionally in the four-year old cambium and none have been found in the cambium of the older stems.

Later in this work we shall deal more fully with the process in the cambium of stems leading to the elimination of fusiform initials. This process has a great influence on anticlinal divisions, which in the stems always occur at the points of the elimination of initials. Besides the strongly oblique pseudotransverse divisions in the long fusiform initials there are also relatively short divisions occurring in groups in the eliminated initials. The various kinds of pseudotransverse divisions in the cambium of older stems are shown in fig. 3.

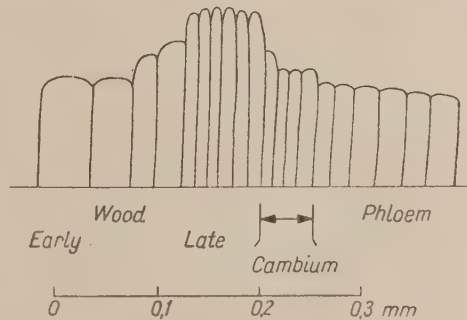
Intrusive growth

Intrusive growth in the fusiform initials can be recognized by comparing the length of these cells with the length of the phloem cells produced previously in the same row, since presumably the phloem cells

do not increase in length during differentiation. The fusiform initials are usually longer than the young phloem cells in the same row and a negative difference of length may occur only in the case of cells just in the course of elimination. The difference between the length of fusiform initials and the length of the young phloem cells in the same row can be, therefore, regarded as an indicator of the magnitude of the intrusive growth which took place in these initials in the time between the formation of the phloem cells concerned and the fixing of the cambium.

Fig. 4 shows the levels of the ends of cells forming a radial row of common origin. The figure illustrates the average conditions in rows not directly neighbouring upon the initials which are eliminated in the cambium of the 80-year old tree. As is to be seen the cambium cells are somewhat longer than the young phloem cells, but somewhat shorter than the

Fig. 4. The ends of cells of common origin forming one radial row in the cambium of an 80-year old tree. The diagram illustrates the average conditions prevailing in rows not neighbouring upon the initials undergoing elimination. The intrusive growth of the fusiform initials is so weak that it is not marked on the diagrams in fig. 5—7. The vertical and the horizontal scales are the same



tracheids of late wood. In the rows neighbouring directly upon the initials undergoing elimination the differences in the length of the phloem cells and of the cambium cells are much greater. The differences in the levels of the ends of the fusiform initials and of the corresponding young phloem cells in the three investigated cambium kinds are shown in figs. 5, 6, and 7. For the particular fusiform initials this difference is represented by an arrow beginning at the level at which the phloem cells end (in the zone of the first ring of aluminous cells) and ending at the level of the tip of the fusiform initial.

As is to be seen there is a considerable variability in the intensity of intrusive growth of the particular fusiform initials in the same cambium. The intrusive growth is specially strong in the initials neighbouring upon the cells undergoing elimination and marked „e“ in the diagrams (the places where the elimination of an initial or its part ended shortly before fixation are marked with broken lines). The three series of photographs in Plates 1, 2, and 3 show the points in the cambium where fusiform initials are being or were eliminated and where the intrusive growth of the neigh-

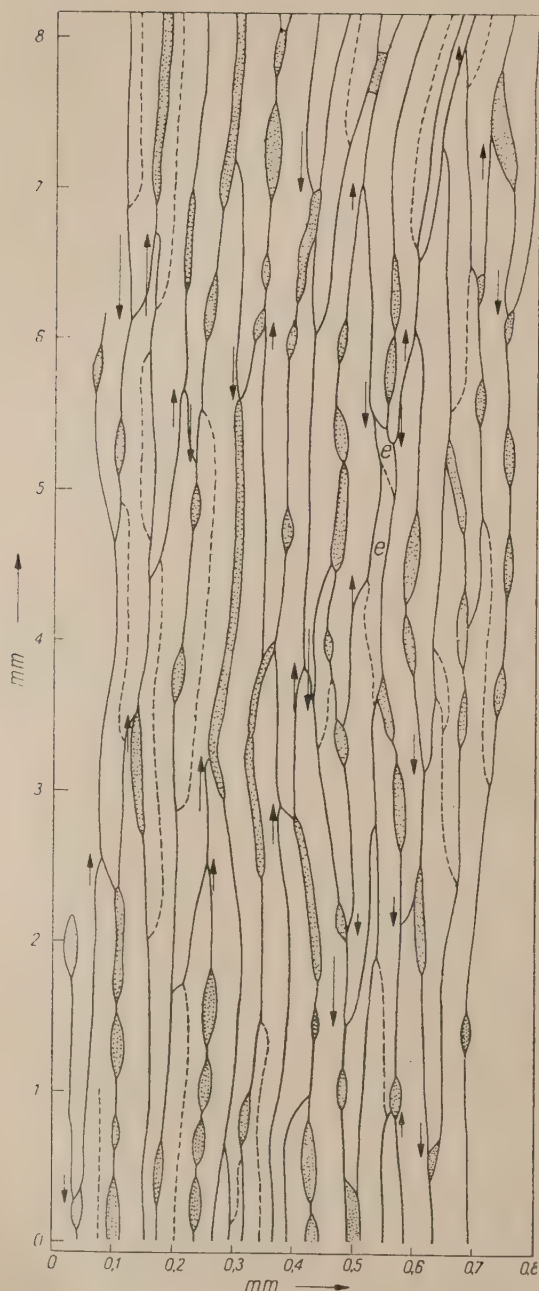


Fig. 5. Reconstructed tangential section through the root cambium. The arrows show the distances between levels of the endings of the fusiform initials and of the corresponding young phloem cells. The arrows beginning at the level of the phloem cell ends extend to the ends of the fusiform initials. In this way the length of the arrows is a relative measure of the intensity of intrusive growth in the fusiform initials. Differences of lengths smaller than 40μ (see fig. 4) are disregarded and the cells showing only such differences are not marked with arrows. The fusiform initials undergoing elimination are marked with the letter "e". The other denotations are the same as fig. 1

bouring cells is very strong. In order to bring out more clearly what is taking place in these places the plates are supplemented with diagrams (figs. 9, 10, and 11) showing the reconstructed tangential sections of the corresponding parts of the cambium in the plane of the fusiform initials and in the plane of the young phloem cells. In each of these figures the diagrams *B* show the state of the fusiform initials at the time when they were fixed and the diagrams *A* the conditions which prevailed in the cambium somewhat earlier at the time of the formation of the phloem layer with the albuminous cells.

The analysis of all these illustrations as well as of photographs of the other parts of the cambium not reproduced in this report leads to the conclusion that the places round the eliminated cells are the centres of especially strong intrusive growth.

The intrusively growing ends of cells force their way inbetween the anticlinal walls of the neighbouring cells. The new walls derived from anticlinal divisions are accessible to this intrusive growth during a short time after their formation. Cell ends have been observed between the walls from anticlinal divisions with only a few periclinal divisions following the anticlinal division.

At this point the question arises what is the direction of overlapping when the ends of two cells growing in opposite directions meet? It seems that in the places where there is no elimination of cells and where the intrusive growth is weak the fusiform initials may overlap either to the right or to the left. However, in the places where an initial is eliminated, and where the intrusive growth is strong, the left-hand shift of the ends is always observed. This state of things is illustrated e.g. by the series of photographs in Plate II (the upper end of the initial „d“ in fig. 11). At the point where an initial is eliminated the direction of intrusive growth of the ends of neighbouring cells is probably determined by the direction of the anticlinal divisions in the eliminated cell.

The intrusive growth is strongest in the cells from the middle of the cambium zone, i.e. in the fusiform initials (see photographs in fig. 8, the level of about 800 μ in series C on Plate I, and the level of about 4440 μ on Plate III).

Mention has been made earlier of intrusive growth derived from a pseudotransverse division. Generally speaking this growth is not noticeable till some time after the anticlinal division, already in the course of the periclinal divisions.

In the root cambium where the elimination of initials is very limited intrusive growth after an anticlinal division is not specially strong. The situation is different in the cambium of stems, where after an anticlinal

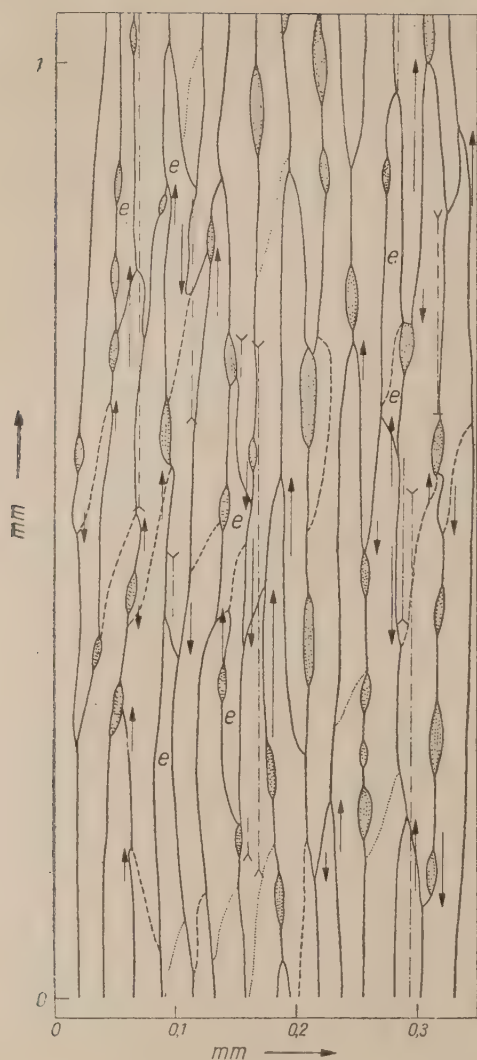


Fig. 6. The same as in fig. 5, but for the cambium from a four year old stem. The dot-dash line marks the place where a fusiform initial or its part was not long ago eliminated from the cambium, i.e. the place where the radial row is interrupted in the cambium zone. The level corresponding to the ends of phloem cells in the interrupted row is marked with the sign v: thus the dot-dash line without the sign v corresponds to the level at which a fusiform initial still not eliminated from the cambium begins and this initial is marked with the letter "e"

division the growth of the newly formed initials often is very strong (series C, Plate I). However, this intense growth is always associated with the elimination of some neighbouring cells, even if it is a sister cell from the anticlinal division. Owing to this circumstance it is difficult to distinguish the intrusive growth following on an anticlinal division from the intrusive growth associated with the elimination of a neighbouring cell.

There is no intrusive growth in the fusiform initials which are in the course of elimination.

No noticeable difference in the intensity of growth of the lower and the upper ends of the fusiform initials has been observed. In some particular initial usually the growth of one end only is intense (if there is any

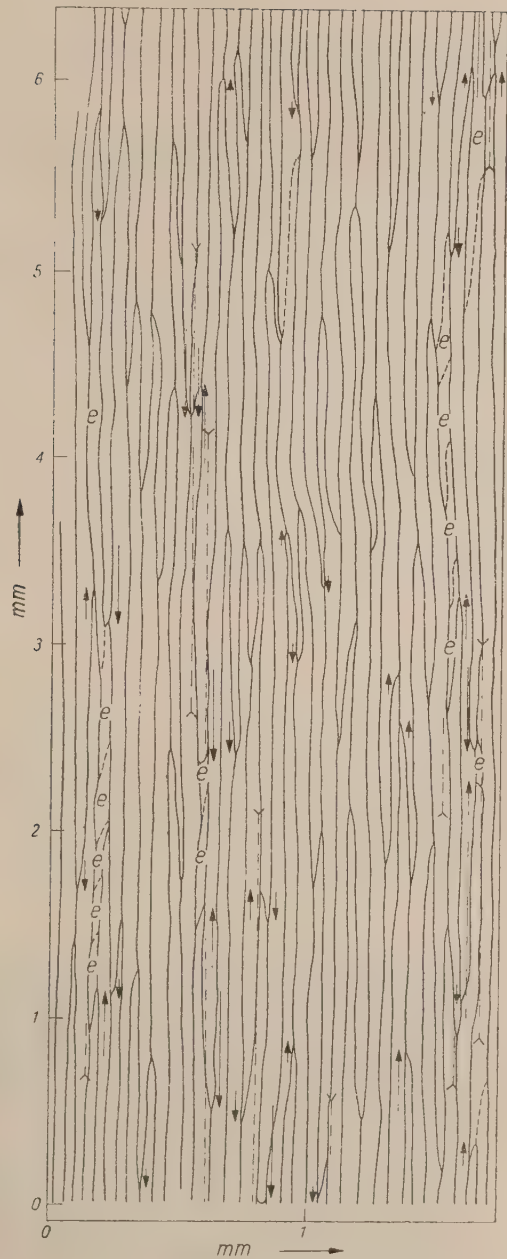


Fig. 7. The same as in fig. 6 but for the cambium from an 80-year old stem

intense growth in the cell at all). The strongly growing end is the one which neighbours with the eliminated cell.

Similarly, no differences have been observed between the ability for intrusive growth of the upper and the lower ends of the xylem mother cells. In the stem of the 80-year old tree intrusive growth associated with

the differentiation of the tracheids of late wood is about 80μ at each end, whereas the length of the cells is 4.5 mm.

The intrusive growth of the fusiform initials in the cambium of the root and of the young stem is on the whole stronger than in the cambium of the older stems, which is clearly apparent when the lengths and the number of the long arrows representing the intrusive growth in figs. 5, 6, and 7 are compared. In the cambium of the older stems intrusive growth tends to be concentrated in the places where initials were eliminated, whereas in the young stem the growth of most initials is rather strong even when there are no visible traces of elimination in the neighbourhood. This difference seems to be associated with the difference in the relative rate of the girth increase in the young stem and the root on one hand and of the older stem on the other. The increase of the girth of the older trees is so slow that probably, if there was no elimination of initials and if the replacement of the eliminated cells was not necessary, the rate of intrusive growth and of the other changes in the cambium would be too slow to be apperceivable.

The elimination of the fusiform initials

The phenomenon of the elimination of initials is common in the cambium of stems and takes place both in the young as well as in the old cambium. However, the elimination is the most distinct in the cambium of old stems, because in the old stems the changes occurring in the cambium are concentrated at the points of the elimination of initials. In the stem of the 80-year old tree there are on the average about 20 cells

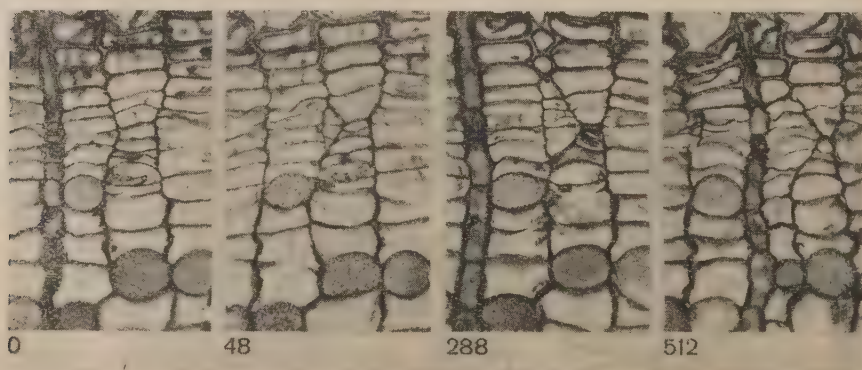


Fig. 8. A series of photographs of the cambium from an 80-year old stem showing a fusiform initial in the course of elimination and the intrusive growth of the neighbouring initial. The figures indicate the distance in μ of the level of each photograph from the level of the first one

in the course of elimination to one centimetre of the circumference of the cambium containing about 200 fusiform initials. In the cambium of the root used for the investigation the elimination of the fusiform initials is very rare.

The phenomenon of the elimination of fusiform initials consists in the breaking of continuity of the radial row of the cambium cells so that the cell which so far was an initial cell, is moved either to the phloem or to the wood (Plates I, II, and III). The process always begins by the narrowing down of the row of cambium cells (fig. 8 and the level of about 3000 μ on Plate III). The narrowing down of a row is accompanied by strong intrusive growth of the ends of the neighbouring cells (i.e. the ends of the cells directly neighbouring upon the cells undergoing elimination); the intrusion proceeding on the whole along the radial walls of the contracting row is caused by the cells responsible for the elimination forcing their way sideways inbetween the cells which are to be separated. Even the initial cells of the ray may take part in the sideward pushing apart of the other cells (see e.g. level 2400 μ in series B on Plate I) though it is possible that ray cells play only a passive part under the pressure of the neighbouring fusiform initials.

The elimination may affect one of the cells derived from a pseudotransverse division of a long fusiform initial (then the other cell grows strongly at the newly formed end and takes part in the elimination of its sister cell), or it may affect a whole initial which does not begin to divide anticlinally into several segments till after it is submitted to sideward pressures. The final break of the continuity in a radial row of cambium cells (divided anticlinally into segments) takes place in stages in such a way that the pushing apart affects one segment. Cells of each segment are forced apart over the whole of their length simultaneously. The sequence of the anticlinal divisions in the fusiform initials in process of their elimination is such that the initial is first divided into long but not necessarily equal segments and then each part is again divided into smaller segments. This is illustrated by the group of anticlinal divisions in row „e“ of series B on Plate I.

DISCUSSION

The results of this investigation may help in the understanding of the processes taking place in the nonstoried cambium, though on the whole these processes are known. The problems considered are not new, but the method applied in this work of directly observing the cambium itself was new. Owing to this many details of the investigated phenomena have appeared more clearly than in the earlier researches. Among the new

details there is an important one: the divisions taking place in the cambium of larch all have one direction. We may as well start the discussion with this trait.

The earlier investigations carried out by Bannan and his school (Bannan, Whalley, Bayly) have brought no decisive information as to the direction of the anticlinal divisions. It is impossible to say whether in the species studied by that school (larch was not one of them) the anticlinal divisions were one-directional or not (it is noteworthy that in the root cambium of larch the anticlinal divisions are not one-directional). This trait might have occurred in those species, but it was not noticed when the history of the development of cambium was reconstructed from the tangential sections of the xylem or the phloem. Although, on the few drawings illustrating the reports of Bannan's school walls from anticlinal divisions can be seen running from right to left or in the opposite direction, it is impossible to decide how far these diagrams are comparable because, apart from other reasons their orientation is unknown (for a given direction of the rotation it is not the turn in the plane of the cambium that is important but the side from which this plane is observed).

The question thus arises, what may be the significance of the fact that the divisions in fusiform initials are one-directional?

As is well known, the secondary wood elements are often arranged in a spiral. This is clearly visible in the trunk from which a long splinter was cut out by lightning. The spiral arrangement of fibres is a well known defect of timber when the spiral is so compact that it makes difficult the working of the wood. The causes of the spiral arrangement of fibres in timber are unknown (Brown et al. 1949). According to Champion (1925) compact spiral may be a hereditary trait. It seems probable that in general the spiral arrangement is a characteristic trait of an individual or of a species, but the normal spirals are too loosely wound to be easily noticeable.

Examinations of the trunks of the European larch, including also the trees of this investigation, show that the cracks in the rhytidome usually run along a spiral.

The spiral arrangement of cells in the xylem and in the phloem is evidently caused by the spiral arrangement of the fusiform initials in the cambium. Thus, to explain the reason for the presence of spirals in wood it is necessary to explain the circumstances in which spirals are formed in cambium. In this last case the reason for the formation of spirals may easily be the fact that anticlinal divisions are one-directional.

The significance of one-directional anticlinal divisions in the formation of the spiral arrangement is obvious. Their effect can be either direct,

by determining the direction of the walls formed in the course of anticlinal divisions, or indirect, by determining the direction of intrusive growth which is another source of anticlinal walls. It, therefore, seems plausible to suppose that the greater the number of anticlinal divisions with the same direction of rotation and the stronger the intrusive growth accompanying these divisions, the greater will be the probability for the formation of a spiral arrangement.

If the frequency of the anticlinal divisions and the intensity of intrusive growth were only just sufficient to increase the girth of the cambium, then the development of a spiral arrangement would be less probable. However, the elimination of the fusiform initials may be considered to be the mechanism accelerating the rate of the changes in the cambium. Owing to the elimination of these cells both intrusive growth and anticlinal divisions may take place more rapidly than what is indicated by the rate at which the distance of the cambium from the centre of the trunk increases.

According to Bannan the elimination of the fusiform initials reflects the competitive conditions prevailing among the initials and acts as a mechanism of selection. In his opinion the most successful initials in the competition are the longest ones and the ones having the greatest number of contacts with the rays. In his later reports Bannan considered this selection to be a part of the mechanism responsible for the well known regular changes in the length of the xylem elements.

However, it seems that selection is not the only motive for elimination of fusiform initials. The results of the present investigation indicate that the elimination effect plays a part in accelerating the rate of intrusive growth and of the anticlinal divisions in cambium allowing the rearrangement of the fusiform initials and thus of wood. This role of the elimination would then have a special significance in the formation of the spiral arrangement, which for mechanical and also physiological reasons can be sometimes more effective than the parallel one.

There is still another question worth considering here. On the ground of statistical analyses Bannan concluded that the growth of the fusiform initials newly formed in the course of an anticlinal division followed a familiar growth patten in which the early rapid extension gave way to a gradually decreasing rate of growth as lengthening proceeded. But the results of this investigation indicate that the rapid growth takes place only in the neighbourhood of an eliminated initial. Since such eliminations often take place in the neighbourhood of the fusiform initial which divides anticlinally or one of the daughter cells is eliminated, it is obvious that the maximum of the intrusive growth rate must statistically occur in

the initial stage of the development of a cell formed in an anticlinal division.

It is impossible to say at present how far this is caused by some inner rhythm in the cell development and how far it is the result of the influence derived from the cell undergoing elimination, but the latter factor should not be disregarded. There is, of course, the possibility of reversing the whole problem by saying that it is the capacity of a cell for rapid growth after an anticlinal division which causes the elimination of a neighbouring cell. However, it is necessary to keep in mind that the anticlinal division of a cell not necessarily must imply the rejuvenescence of a cell. As has been demonstrated, it may have a contrary effect if the division occurs in the course of the elimination of a cell.

CONCLUSION

This paper reports on an investigation carried out on a series of sections through the cambium of *Larix europea* from the root and from the stems of different age. All the changes taking place in the cambium described by Bannan and his school from examinations of the phloem and the xylem have been confirmed, i.e. intrusive growth, anticlinal divisions, and the loss of fusiform initials. The newly discovered element in these processes is the one-directional character of the pseudotransverse divisions. In the cambium from the stems of larch these divisions are left-hand directed but in the root cambium the directions of the divisions are different.

The results of this investigation lead to the conclusion that the elimination of the fusiform initials constitutes the mechanism accelerating the rate of the changes taking place in the cambium. Owing to the elimination of some fusiform initials the intrusive growth and the anticlinal divisions in the remaining initials can proceed more rapidly than it is indicated by the rate at which the circumference of the cambium increases.

The following hypotheses have been advanced: 1) the one-directional character of pseudotransverse divisions is an important factor of the mechanism controlling the formation of the spiral arrangement of cambium cells and thus also of the spiral grain in wood; and 2) the rate of the structural transformations of the cambium, e.g. the rate at which the spiral arrangement of the cells is formed, is controlled by the intensity of the processes in which the fusiform initials are eliminated.

The technical part of this investigation carried out at the anatomical laboratory of the Department of Dendrology of the Polish Academy of

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Much valuable assistance in the making of sections and the photographs was offered by Miss A. Jachnik and in the making of the drawing by Mr J. Cebrat.

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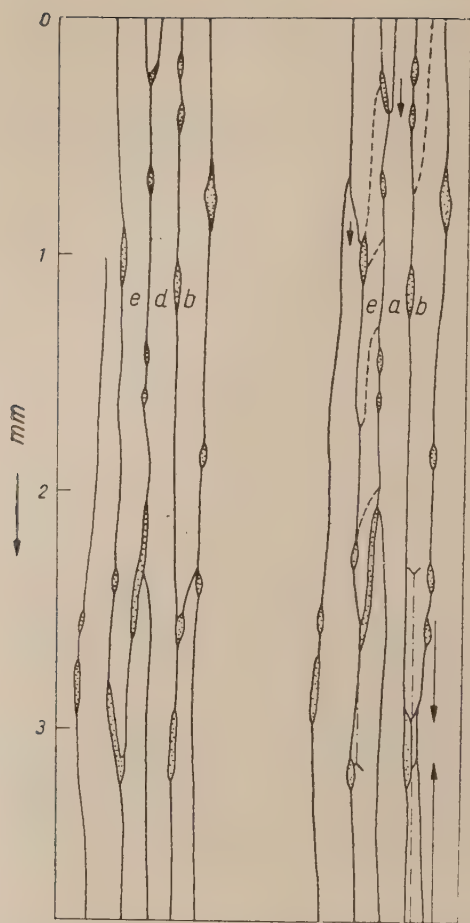


Fig. 9. Reconstructed tangential sections for the fragment of the cambium from which the photographs in series B on Plate I were made. A — reconstructed section in the plane of young phloem, B — reconstructed section in the plane of the fusiform initials. The broken lines represent the walls from anticlinal divisions. The arrows show the intensity of the intrusive growth of the fusiform initials (from the time at which the young phloem was formed to the time of fixation). The dot-dash lines show the places where the elimination of the fusiform initial or of its part has already ended.

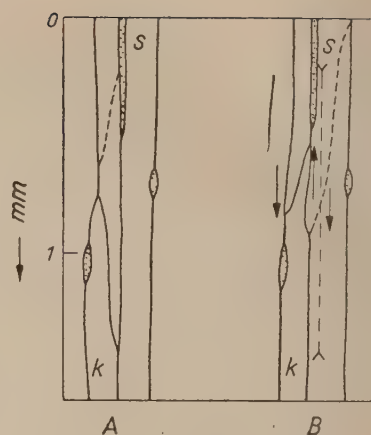


Fig. 10. The same as in fig. 9, but for the series C on Plate I. The diagrams illustrate the intense intrusive growth after an anticlinal division in the neighbourhood of a cell undergoing elimination

Plate I

Three series of photographs of the cambium from *Larix europea*

Series A — root cambium: note the fusiform initials after pseudotransverse division. Series B — cambium from an 80-year old stem: the various anticlinal divisions are visible. The reconstructions of the corresponding tangential sections are shown in fig. 9. Series C — cambium from an 80-year old trunk: the photographs show intense intrusive growth in a fusiform initial neighbouring upon an initial undergoing elimination. The reconstructions of the corresponding tangential sections are shown in fig. 10.

The numbers in the left bottom corner of each photograph give the distance in μ of the level of the photograph from the level of the first photograph in the series. The distance was measured from the top downwards.

Plate I

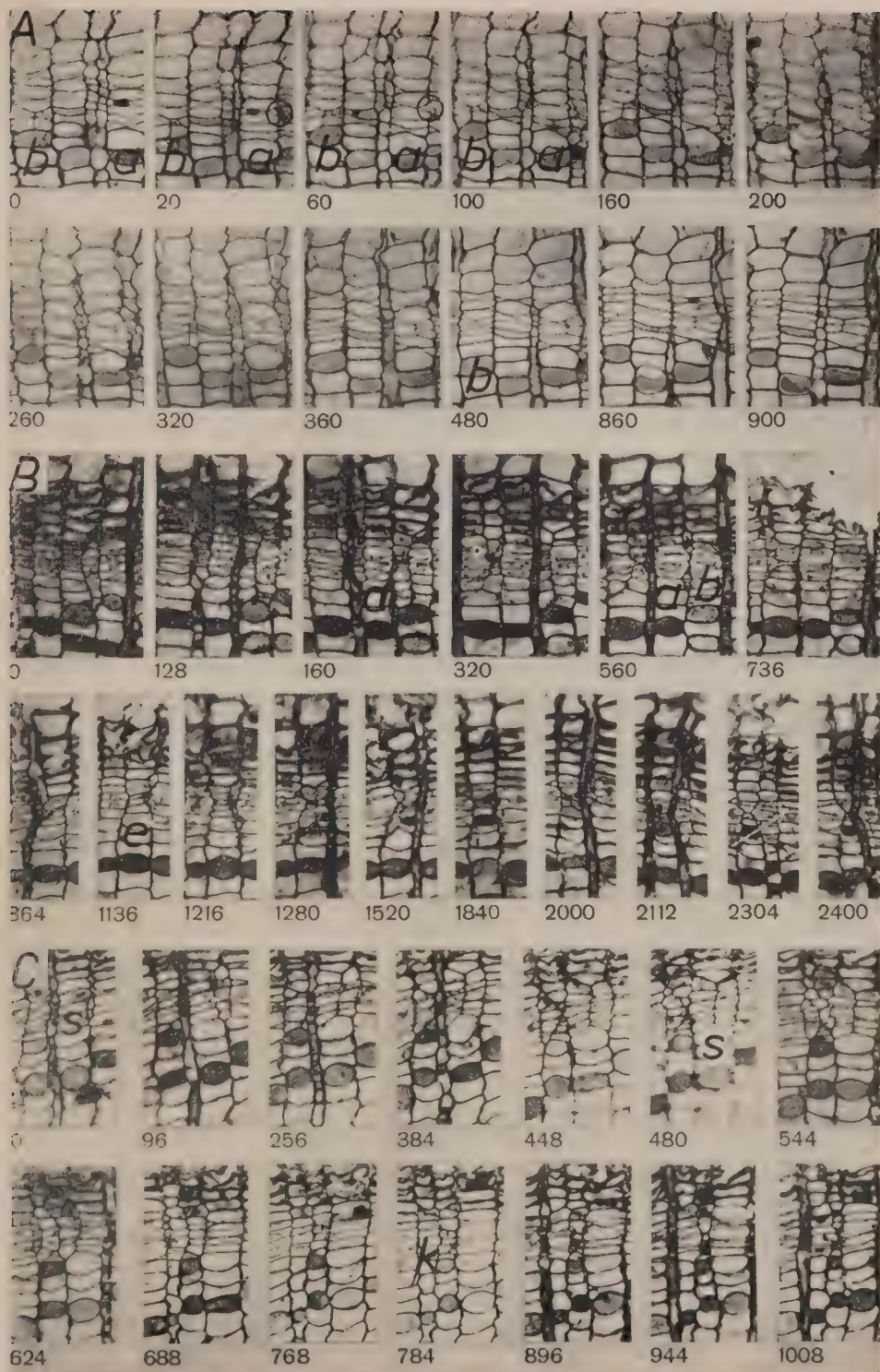
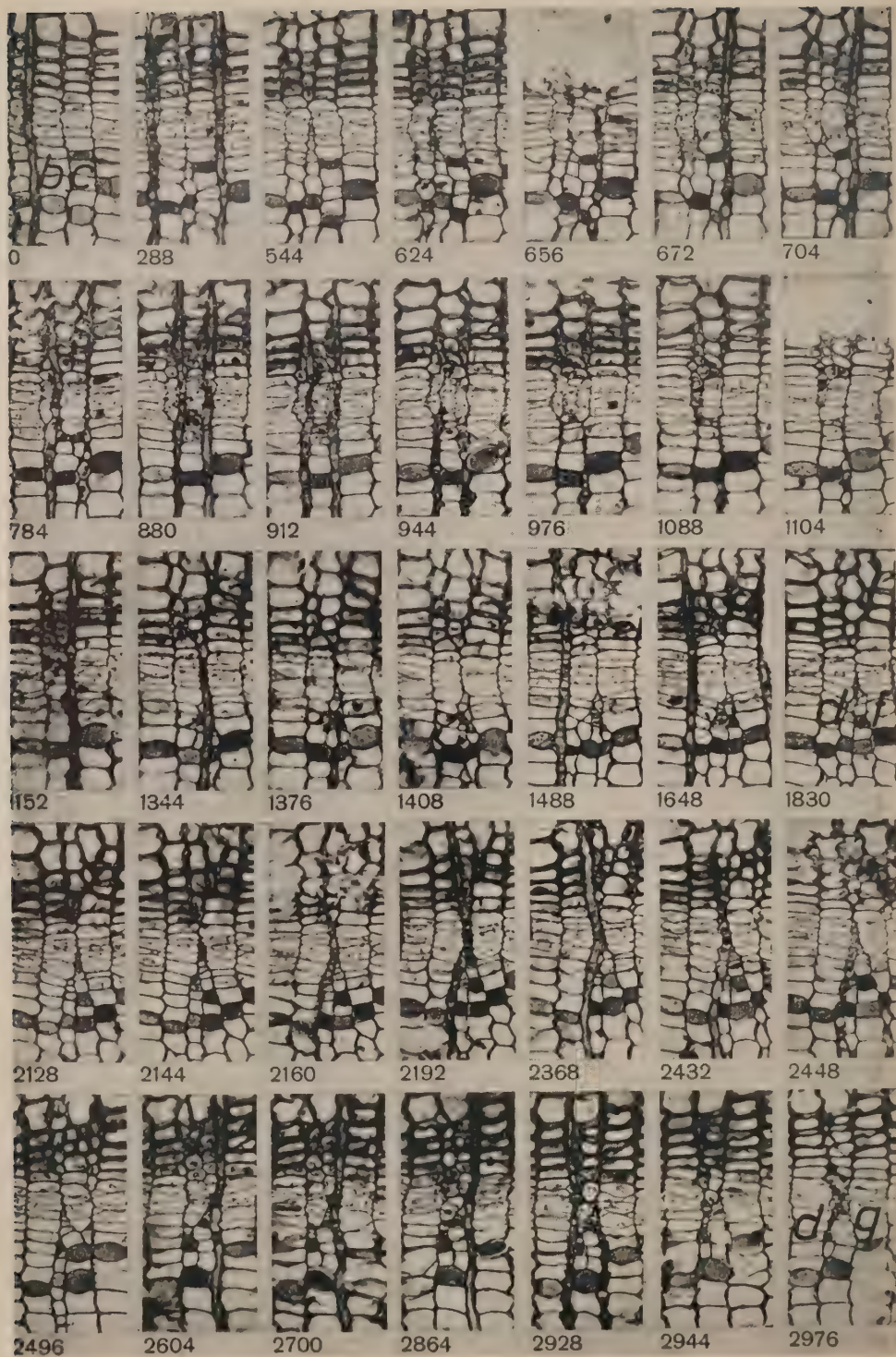


Plate II



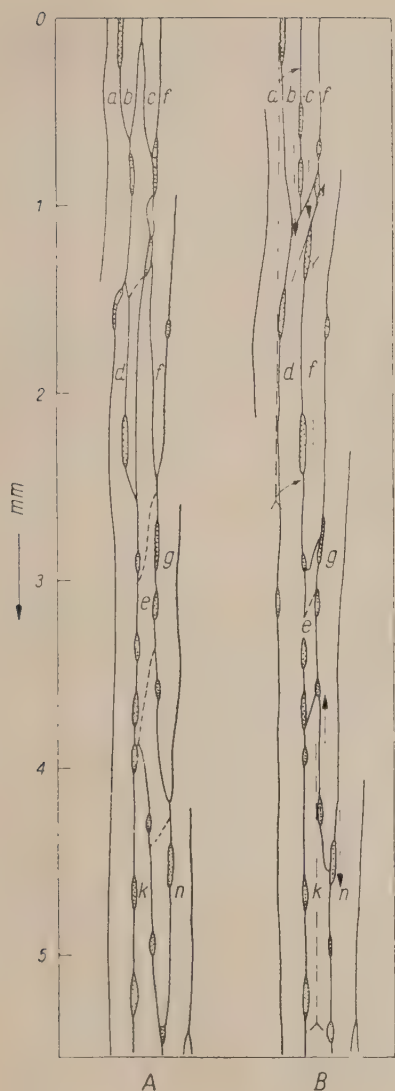
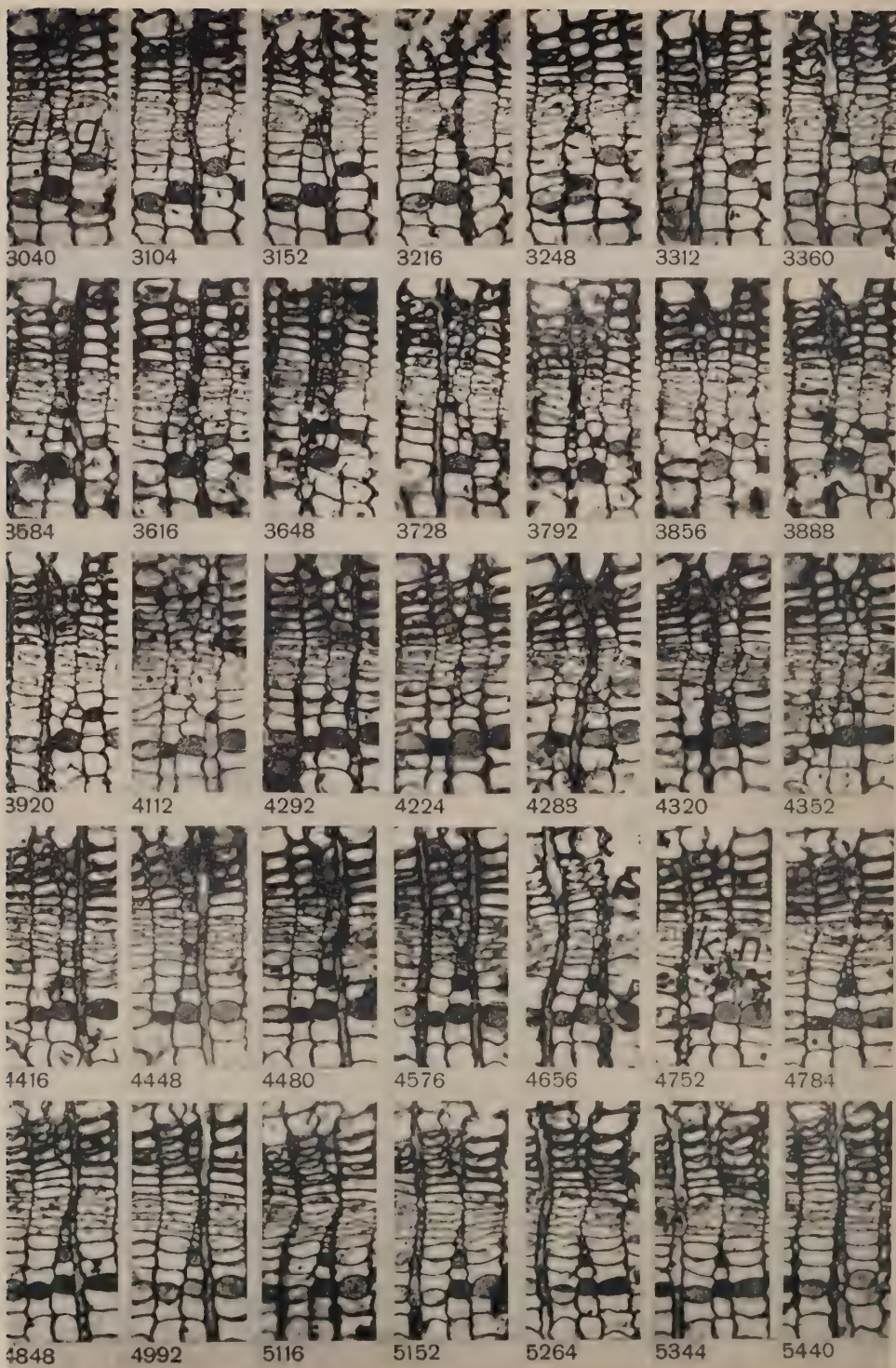


Fig. 11. The same as in fig. 9, but for the series of photographs in Plate II and III. The diagrams illustrate the behaviour of cells at the point where a fusiform initial is being eliminated and in its neighbourhood. Note in particular the intrusive overlapping onto the right side of the cells which the large cell marked "d" meets on its way

Plate II and III

A series of photographs of the cambium from an 80-year old tree showing the conditions prevailing in the place where a fusiform initial is eliminated. The reconstructions of the corresponding tangential sections are shown in fig. 11.

Plate III



Fazy rozwoju woreczka zalążkowego w zestawieniu z rozwojem pyłku u *Lilium candidum*

Phases of embryo-sac development and pollen grains development
in *Lilium candidum*

B. RODKIEWICZ

WSTĘP

Rozwój mikrospor we wszystkich pylnikach kwiatu lili odbywa się w sposób dość zsynchronizowany. W niektórych fazach synchronizacja jest nieomal idealna — stadium I profazy mejotycznej (Tabl. I, fig. 2), stadium tetrad; w innych stadiach synchronizacja jest mniej wyraźna — I i II metafaza, anafazy. Dolna część pylnika zawiera komórki, które czasami rozwijają się nieco szybciej niż w części środkowej i górnej. Pewien stopień synchronizacji daje się również zauważyć w rozwoju zalążków w środkowej strefie zaląźni. Przede wszystkim usiłowano stwierdzić, jaki jest stopień tej synchronizacji, a następnie, czy określone stadium rozwoju pyłku odpowiada określone stadium rozwoju megaspor. Okazało się, że istnieje odpowiedniość niektórych stadiów obu typów komórek. Ponieważ jednak pręciki lili dojrzewają szybciej niż słupek, to bardziej zaawansowanym fazom rozwoju haploidalnych komórek pylnika odpowiadają określone, ale młodsze fazy komórek haploidalnych zalążków w środkowej części zaląźni.

Mikrosporogeneza u lili odbywa się w sposób taki, jak u większości roślin okrytozalążkowych. Rozwój woreczka zalążkowego natomiast przebiega według mało rozpowszechnionego typu *Fritillaria*. Typ ten został po raz pierwszy prawidłowo opisany przez Bambacioni (1928), a następnie przez Coopera (1935) i innych u rozmaitych gatunków *Fritillaria*, *Lilium*, *Tulipa* (Maheshwari 1950), ostatnio zaś ze szczególnym uwzględnieniem roli jąderka przez Flinta i Johansena (1958). Komórka macierzysta megaspor różnicuje się pod skórą zalążka, po podziale redukcyjnym tworzą się cztery jądra megaspor. Ani po pierwszym, ani po drugim podziale nie zakładają się przegrody pomiędzy powstałymi jądrami. Dzięki temu cztery jądra megasporialne są zawarte w jednej wydłużonej komórce (Tabl. II, fig. 11). Wszystkie one biorą

udział w formowaniu woreczka zalążkowego. Proces ten jest dość zawily, przede wszystkim trzy jądra grupują się w części chalazalnej, a jedno pozostaje w części mikropylarnej woreczka (fig. 12). Wszystkie jądra woreczka dzielą się, ale sześć grup chalazalnych chromosomów zlewa się w dwa jądra triploidalne. W rezultacie powstaje wtórne czterojądrowe stadium rozwoju woreczka zalążkowego (fig. 13). Po jeszcze jednym podziale powstaje ośmiojądrowy woreczek zalążkowy, w którym aparat jajowy i jedno jądro biegunowe jest haploidalne, a drugie jądro biegunowe i antypody są triploidalne. Fig. 14 przedstawia centralną komórkę woreczka z obu jądrami biegunowymi. Mogą istnieć odchylenia od przedstawionego wzoru uzależnione od stopnia poliploidyzacji i odmiany rośliny (Darlington i La Cour 1940).

METODY

Zalążnie i pylniki *Lilium candidum*, rosnącej w ogrodzie, utrwalano w mieszaninie alkoholu etylowego i kwasu octowego (3 : 1) i przechowywano w 70% alk. etyl. Dla zbadania dużej ilości zalążków i oceny stadiów rozwojowych woreczków zalążkowych najwygodniejszą wydawała się metoda preparatów gniecionych (wg Darlingtona i La Coura 1947). Metodę tę stosowali McLeish i Snod (1959) przy fotografowaniu makrosporogenezy lilii. Całe zalążnie, albo ich części, hydrolizowano zimnym 1n HCl w ciągu kilku minut. Część materiału hydrolizowano na gorąco i przeprowadzano reakcję Feulgena in toto. Po odczynniku Schiffa materiał płukano w trzech zmianach płynu do płukania po 10 min w każdej. Po reakcji Feulgena lub po hydrolizie na zimno wyrastające szeregiem zalążki oddziela się łatwo od reszty tkanek zalążni. Młode zalążki, trudne do odróżnienia, wypreparowuje się razem z łożyskiem stosując słabe powiększenie mikroskopu. Odcięte zalążki rozgniata się w kropli 40% kwasu octowego. W preparatach otrzymuje się zawiesinę grup komórek i pojedynczych komórek, wśród nich wyróżniają się wymiarami komórki macierzyste megaspor. Wysychaniu preparatu zapobiega dodanie gliceryny. Część preparatów podbarwiano dodatkowo zielenią jasną (mieszanina zieleni z gliceryną i kwasem octowym).

Aby ustalić rozmieszczenie różnych stadiów rozwojowych woreczków zalążkowych rozcinano zalążnie poprzecznie na trzy części. Część dolna stanowiła 1/4 całej długości zalążni, część środkowa połowę, a część górna znów 1/4 długości zalążni. W preparatach z każdej części liczono poszczególne stadia rozwoju woreczka zalążkowego.

Macierzyste komórki megaspor w okresie różnicowania się i I profazy (fig. 3—9) podzielono na grupy w zależności od wymiarów (tabela).

Następne stadia wyróżniano na podstawie ilości jąder. W preparatach z młodych zalążni, gdzie odbywa się megasporogeneza, komórki pozostawały nie uszkodzone i łatwe do sklasyfikowania, natomiast w preparatach ze starszych zalążni część woreczków nie oddzielała się od zalążków, bądź była uszkodzona przy rozbijaniu tkanek. Ilość nie oznaczonych stadiów wynosiła wtedy 10—20%.

WYNIKI

Megasporogeneza, a następnie rozwój woreczków zalążkowych, nie odbywa się jednocześnie we wszystkich zalążkach jednej zalążni. Część dolna zalążni posiada zalążki opóźnione w rozwoju. Komórki sporogenne i woreczki zalążkowe w zalążkach części środkowej zalążni dojrzewają najszybciej i w sposób najbardziej wyrównany. Zalążki w części górnej również opóźniają się w rozwoju, ale w mniejszym stopniu niż zalążki dolnego odcinka zalążni. Jeśli więc porównać stopień rozwoju zalążków w całej zalążni, to znajdzie się tam różnorodne stadia. Np. w zalążni o długości 7—8 mm najliczniejsza jest grupa komórek macierzystych megaspor w późnym stadium I profazy. Krańcowo niedorozwinięte są zalążki z różnicującą się komórką macierzystą megaspor, a najbardziej rozwiniętymi są zalążki z czterojądrowymi woreczkami zalążkowymi. W zalążni o długości 11 mm najliczniejszymi są czterojądrowe woreczki, a krańcowymi sporogenne komórki w późnym stadium I profazy i ukształtowane woreczki zalążkowe.

W preparatach wykonanych ze środkowego odcinka zalążni uwidacznia się dość wyraźnie wyrównanie stadiów rozwojowych zalążków. Stadia wcześniejsze obejmujące okres megasporogenezy są lepiej zsynchronizowane od stadiów rozwoju woreczków zalążkowych. Synchronizacja zaznacza się szczególnie w profazie mejozy i I i II stadium czterojądrowym woreczka zalążkowego. Wtedy ponad połowa zalążków jest w tym samym stadium rozwoju. Należy przypuszczać, że wiąże się to z długotrwałością tych stadiów. Inne krótsze fazy nie są zsynchronizowane, odnosi się to głównie do okresu od metafazy I do utworzenia się pierwotnego czterojądrowego woreczka. Z pewnym przybliżeniem można więc mówić o synchronizacji niektórych stadiów rozwoju zalążków znajdujących się w środkowym odcinku zalążni.

Fakt ustalenia częściowej synchronizacji rozwoju zalążków pozwolił na szukanie równoległości rozwoju gametofitów w zalążni i pylnikach. Wiadomo bowiem było, że wiele stadiów rozwoju pyłku odbywa się równocześnie we wszystkich pręcikach kwiatu. Porównywano stadia megasporogenezy i mikrosporogenezy w odpowiadających sobie zalążniach i pylnikach wyciętych z paków kwiatowych różnej wielkości. Określano

i liczono stadia megasporogenezy w środkowym odcinku zalążni (około 1/2 długości całego organu). Stadia mikrosporogenezy określano w preparatach ze środkowej części pylników. Część środkową brano dlatego, ponieważ czasami obserwuje się szybszy rozwój dolnej części w porównaniu z wierzchołkiem. Rezultaty są przedstawione na tabeli 1. Wyróżnicowanie się komórki macierzystej megaspor (fig. 3) i mikrospory (fig. 1) odbywa się mniej więcej w tym samym czasie, w zalążniach o długości około 3 mm i w pylnikach o długości około 4 mm. Jednak tempo rozwoju komórki macierzystej woreczka zalążkowego jest bardzo powolne w porównaniu z mikrosporogenezą. Podczas gdy komórki macierzyste megaspor dochodzą do końca I profazy mejotycznej, w pylnikach zostaje ukończona mikrosporogeneza. Proces przygotowywania się komórki macierzystej megaspor do podziału redukcyjnego wiąże się z ogromnym zwiększeniem jej wymiarów i wytworzeniem dużych ilości cytoplazmy. Wydaje się, że komórka macierzysta w okresie I profazy nagromadza przeważającą część protoplazmy późniejszego woreczka zalążkowego.

Ponieważ mikrosporogeneza kończy się przed ukończeniem I profazy w komórce macierzystej megaspor, należało początkowy okres megasporogenezy podzielić na kilka odcinków. Następnie dla wyznaczonych klas komórek megasporogennych w okresie od początku różnicowania się do końca I profazy trzeba było znaleźć równoczesne z nimi stadia mikrosporogenezy. Najłatwiejszym kryterium podziału była długość rozwijających się komórek macierzystych. Na podstawie różnic długości podzielono je na 7 grup (tabela 1). Jedynym często spotykanym i od razu rzucającym się w oczy stadium profazy mejotycznej było sinapsis (fig. 8). Komórki w tym stadium trafiały zwykle do grupy o największych wymiarach (110—160 μ).

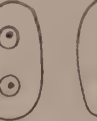
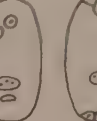
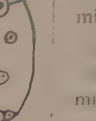
Rozwój woreczka zalążkowego odbywał się w okresie, gdy w pylnikach znajdował się dojrzały dwujądrowy pyłek. O stadium rozwoju woreczka zalążkowego można się było zorientować tylko w przybliżeniu na podstawie wielkości zalążni.

STRESZCZENIE I WNIOSKI

U lilii rozwój komórek podczas mikrosporogenezy jest w znacznym stopniu zsynchronizowany. Megaspory i woreczki zalążkowe rozwijają się nierównomiernie. Najszybciej dojrzewają te, które znajdują się w środkowej części zalążni. Wykonywano preparaty gniesione z zalążków środkowego odcinka zalążni (około 1/2 całej długości zalążni). W tych zalążkach porównywano stadia macierzystych komórek megaspor i woreczka zalążkowego. Obserwuje się pewną synchronizację niektórych stadiów

Tabela I — Table I

Porównanie faz rozwojowych woreczka zalążkowego w środkowej części zalążni z fazami rozwoju pyłku u *Lilium candidum*
 Comparison of developmental stages of embryo-sac in median part of ovary with developmental stages of pollen grains in *Lilium candidum*

Comparison of developmental stages of embryo sac in middle part of ovary with developmental stages of pollen grains in anther														Stadium mikrosporo- genezy Stage of microsporo- genesis	Długość pylnika w mm Length of anther. mm
Stadium megagametogenezy Stage of megagametogenesis															
Nr fotografii (Tabl. I) No. of photographs (Plate I)		3,4		5	6		7	8,9	10	11	12	13	14		
Długość kom. mac. megaspor., μ Length of megaspore mother cell, μ		30	40	60	75	90	110	160							
Długość zalążni w mm Length of ovary, mm														działający się archespor divisions of sporogenous cells	4
3—4		28												początek me- jozy beginning of meiosis	4
3—4		10	43											początek me- jozy beginning of meiosis	5
5			22	38										pachyten pachytene	7
5				20	37									diakineza diakinesis	9
6				35	42									metafaza I metaphase I	10
6				11	51									telofaza II telophase II	11
6					12	34								tetrady microspore tetrads	12
7					17	35								tetrady microspore tetrads	11
7						18	34							tetrady microspore tetrads	13
7							15	26						mikrospory microspores	16
8							16	31						mikrospory microspores	15
8							17	42						mikrospory microspores	16
9							1	31	6	1				dwujadrowy pytek two-celled pol- len grains	17
10								22	7	4				dwujadrowy pytek two-celled pollen grains	18
11									4	6	38	18	6	dwujadrowy pytek two celled pollen grains	20
12										2	25	15	5	dwujadrowy pytek two celled pollen grains	20
13											5	33	3	dwujadrowy pytek two celled pollen grains	21

rozwojowych zalążków. Stopień synchronizacji jest prawdopodobnie tym większy im dłużej trwa dane stadium. Istnieje odpowiedniość pomiędzy fazami mikrosporogenezy i megasporogenezy, ale nie udało się ustalić ścisłej równoległości poszczególnych stadiów. Jednak na podstawie stadium rozwojowego pylnika można w przybliżeniu ustalić stadium rozwojowe zalążków w środkowej części zalążni. Rozwój komórek macierzystych mikro- i megaspory zaczyna się prawie jednocześnie, ale kiedy komórka macierzysta makrospor kończy profazę pierwszego podziału mejotycznego, w pylniku znajduje się dojrzały pyłek.

Katedra Anatomii i Cytologii Roślin UŁ
Łódź

(Wpłynęło: 17.3.1961 r.)

SUMMARY

The course of microsporogenesis in *Lilium candidum* is synchronised to a large extent. The megaspores and embryo sacs develop unevenly. The earliest to ripen are those situated in the middle of the ovary. The respective stages of development of the mother cells of embryo-sac and of the embryo-sac itself in the central part of the ovary (approx. half-way its length) were compared by means of smear preparations. A certain degree of synchronisation in the development of the respective stages, probably proportional to some extent to their duration, was observed.

There is definitely some correspondence between the different phases of mega- and microspores but no strict parallelism was found. The development of mother cells of micro- and megaspores starts almost simultaneously but while the megaspore mother cell is at the end of first division of meiosis, pollen grains in the anther are already mature.

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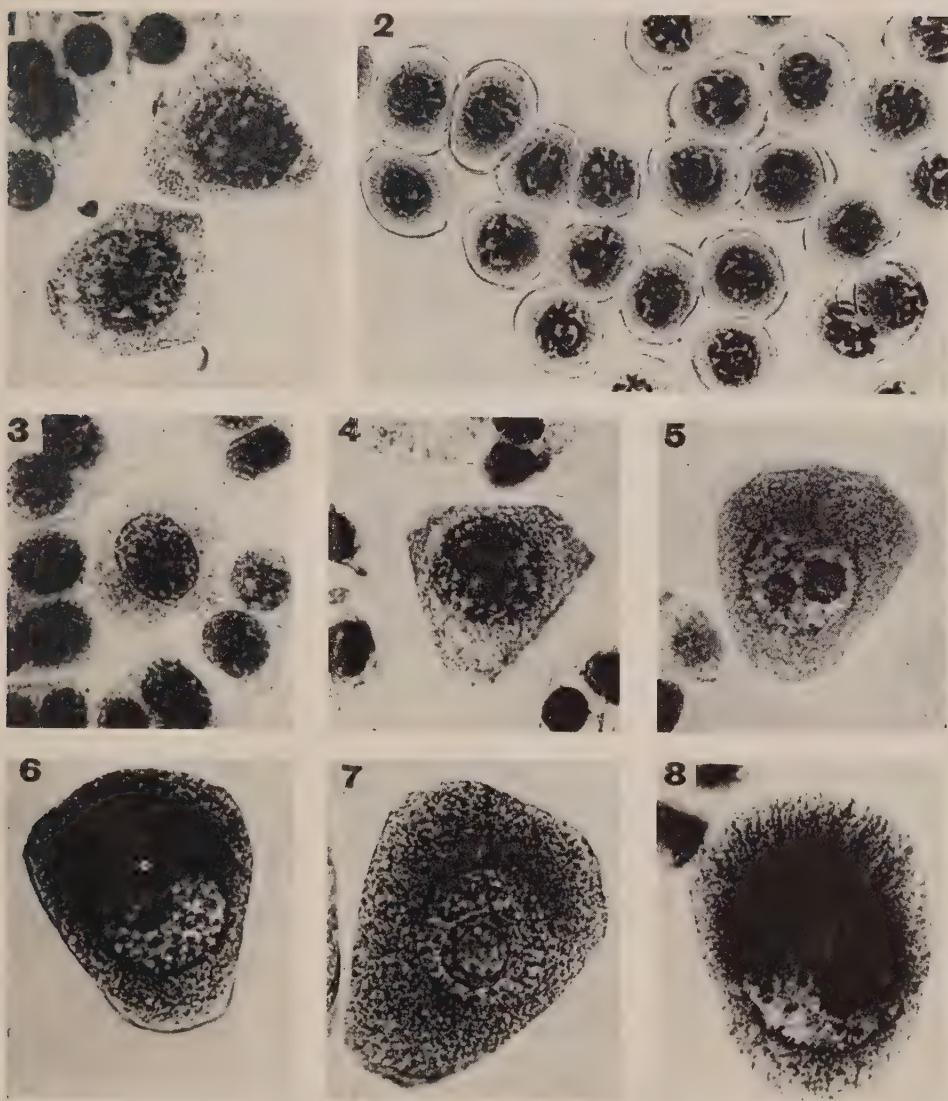
Objaśnienia do tablic I—II
Explanations of the plates I—II

Komórki *Lilium candidum*
Cells of *Lilium candidum*

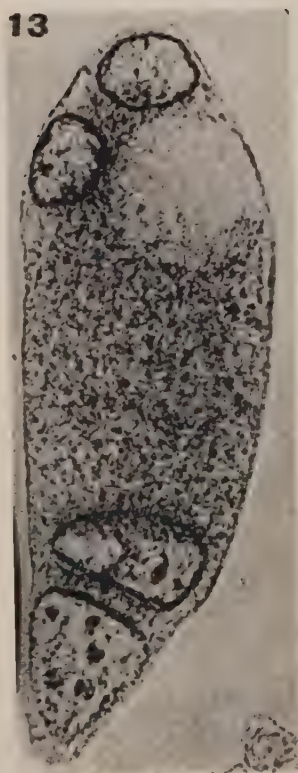
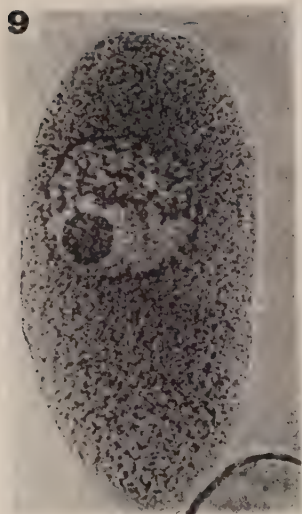
- Fig. 1. Początek mejozy w komórkach macierzystych mikrospor
The beginning of meiosis in microspore mother cells
- Fig. 2. Zsynchronizowana profaza (diakinaza) mejozy w pylniku
Synchronised prophase (diakinesis) of meiosis in anther
- Fig. 3—9. Różnicowanie się komórki macierzystej mikrospory
Differentiation of microspore mother cell
- Fig. 10. Dwa jądra po pierwszym podziale redukcyjnym
Two nuclei after first reduction
- Fig. 11. Cztery jądra makrospor — I czterojądrowy woreczek zalążkowy
Four macrospore nuclei — primary 4-nucleus embryo-sac
- Fig. 12. Trzy jądra chalazalne i jedno mikropylarne w woreczku zalążkowym
Three chalazal and one micropylar nuclei in embryo-sac.
- Fig. 13. Wtórne czterojądrowe stadium woreczka zalążkowego, dwa duże jądra triploidalne
Secnodary 4-nucleus embryo-sac stage, two large triploidal nuclei
- Fig. 14. Część dojrzałego woreczka zalążkowego z dwoma jądrami biegunowymi
Part of mature embryo-sac with two polar nuclei

Wszystkie zdjęcia (z wyjątkiem fig. 2) tak samo powiększone
The same magnification of all photographs (except fig. 2)

Tablica I — Plate I



Tablica II — Plate II



On the photoperiodic control of extension growth and wood formation in Norway spruce (*Picea abies* (L.) Karst.)

T. WODZICKI, L. WITKOWSKA

Investigations on the photoperiodic control of growth and annual ring differentiation of larch and spruce seedlings showed that under short day conditions, extension growth was inhibited and thick-walled tracheids were formed (Żelawski 1956, Żelawski, Wodzicki 1960). In further experiments with larch (Wodzicki 1961a) both appearance of terminal buds and first full differentiated thick-walled tracheids were observed after about 20 short photoperiods. In spite of this correlation, investigation with low intensity light treatment during 12 night hours showed that thick-walled tracheids formation in larch seedlings was probably not dependent on the cessation of extension growth (Żelawski 1957, Molski, Żelawski 1958, Wodzicki 1961a).

Moreover, it was observed in 1-year old larch that if only the top of main shoot was covered (short-day treatment) and all other aerial part of plant subjected to continuous illumination, only the thin-walled tracheids were formed both in plants with lateral shoots decapitated or intact (Wodzicki 1961a). These experiments seem to provide further evidence of the absence of direct connection between photoperiodically controlled processes of extension growth and the thickening of cell walls of tracheids in larch. The conclusion is of great importance for the study of the mechanism of annual ring differentiation in larch and should be further investigated in other conifers.

The above mentioned experiments with only the top of main shoot covered also showed that the photoperiodic reaction bringing about the cessation of extension growth and formation of resting bud in larch was limited only to the shoot under short-day treatment since the lateral shoots under continuous light continued to grow. In this case, photoperiodic stimulus inducing the rest was perceived by young, growing needles or by the shoot apex itself and was not transported to lateral shoots.

The scanty experimental data concerning the locus of photoperiodic perception in relation to dormancy mainly refers to some deciduous

species (Wareing 1954, Van der Veen 1960) but the problem seems to be very interesting in the light of general discussion on the locus of dormancy in trees.

The present paper reports on some additional results of experiments on Norway spruce with reference to two questions:

- a) locus of photoperiodic perception in relation to the rest of shoot apex,
- b) influence of the photoperiodically controlled extension growth on the kind of wood formed.

METHODS

Three-year old *Picea abies* (L.) Karst. plants potted in Wagner's pots were used in the investigation carried out in 1961. Prior to the experimental treatment all plants grew 62 days under continuous illumination and then were subjected to one of the following treatments in a room specially adopted to photoperiodic experiments:

- a) continuous illumination
- b) short-day (12 hours darkness during the night)
- c) continuous illumination and only the top of main shoot covered (the top of shoot under short-day treatment)
- d) continuous illumination, top of the main shoot covered and lateral shoots decapitated
- e) continuous illumination, lateral shoots decapitated.

The first four combinations consisted each of three plants and the fifth of two plants. The top of main shoots were covered with $40 \times 10 \times 36$ mm boxes made of light-proof photographic paper — black on one side and silver on the other — as in earlier experiments with larch (Wodzicki 1961a). During the experimental treatment all the newly appearing buds in two decapitated groups of plants were systematically removed at an early stage of development. After the 30 days of experimental treatment all the plants were subjected to continuous illumination for 20 days and then harvested for anatomical examination.

Continuous illumination, about 4500 lux, was provided by the system of white-light fluorescent "TELAM" tubes. The daily temperatures were nearly constant: mean daily maximum — 23.6°C , mean daily minimum — 22.0 . The daily air moisture ranged on the average from 67.7 to 77.0%.

For the sake of comparison, the wood of three comparable spruce plants grown in natural conditions in Experimental Forests in Rogów was also examined.

All plants were killed and preserved in ethyl alcohol. Transversal sections from the basal part of plants were cut by hand. The method

of preparation and staining with safranin and light-green was the same as used in the earlier investigations with larch plants growing in natural conditions (Wodzicki 1960). Radial diameter and lumen of tracheids were measured in two transversal sections of each examined plant. Thickness of cell walls was calculated as a difference of radial diameter and lumen of tracheids. * Tracheids were measured in the area of newly formed ring of wood along two radiuses perpendicular to each other. Thus, the wood in each of the first four groups of plants (a, b, c, d) and of plants grown in natural conditions were characterized by 12 rows of figures and the plants of the last group (e) by 8 rows. One of the rows of figures obtained from the measurement of tracheids in plant that were submitted to short-day conditions is set below to illustrate the method:

	21**	21	24	18	19	17	11	15	20	20	22	25	27	
cambial zone														previous year late wood
	8***	8	8	8	6	6	6	5	4	3	4	4	4	

(the values are given in micrometer units; one micrometer unit = 0.7 microns)

Wood that had formed during the first period of growth under uniform conditions of uninterrupted illumination was characterized in all groups by the radial diameter and cell-wall thickness of the third, fourth and fifth tracheids counting from the previous year late wood. In plants under short-day treatment, thick-walled tracheids occurred in the outer part of newly formed wood. In order to characterize the wood in this zone, the dimensions of the last three tracheids were taken into account. Simultaneously, in all other groups of plants, the mean dimensions of the last three tracheids were considered as characteristic for the wood formed under various experimental treatment.

The early wood of plants from natural conditions was characterized by the radial diameter and cell-wall thickness of the third, fourth, fifth and sixth tracheids as from the boundary of previous year annual ring of wood in radial direction. Late wood was represented by the size of third, fourth, fifth and sixth tracheids as from the cambium after the annual ring formation was completed.

The method of characterizing the wood formed under various experimental conditions on the basis of the average sizes of a few selected tracheids of each zone was applied and tested in earlier experiments with larch and proved most convenient for this kind of investigation (Wodzicki 1961a).

* Cell-wall thickness of each tracheid represents the total thickness of two tangential cell-walls.

** Radial diameter of tracheids.

*** Cell-wall thickness of tracheids.

RESULTS

Extension growth of plants under continuous illumination was continuous except for one short period of cessation of growth which occurred at different times in most plants of groups *a*, *c*, and *e*. This inhibition was manifested by the formation of a few light-green, scale-like needles at the top of lateral or main shoots but no resting-buds were formed and, in all cases, the growth was resumed within 2 to 7 days. It is noteworthy, that this temporary depression of growth in some plants did not occur at the same time in different shoots. Such a pattern of intermittent growth was also observed earlier in larch under continuous illumination (Żelawski 1956) and in some other woody species (the literature reviewed by Wareing 1956, Nitsch 1957) and it did not influence much the results of present investigations.

Five of the plants with the top of main shoot covered formed the resting bud after 22—25 days and only one after 29 days. At the very beginning of the period of keeping the top of shoot under cover, the last plant (of group *c*) had just interrupted its growth, as described above, and in spite of the cover, the main shoot began to grow a few days later. Finally, the growth of this plant stopped and the resting-bud formed but with some delay as compared with the other plants. This observation gives ground for assuming that the reaction of that plant as being similar as of other plants in groups *c* and *d* in which the top of main shoot was covered.

Short-day treated plants (group *b*) formed the resting-buds at the top of main shoot at different times than at the top of the lateral shoots. The buds at the top of lateral shoots situated lower down began to form after 10 to 12 short photoperiods and at the leaders after 21 to 23 short days.

As mentioned above, all the plants were submitted to continuous illumination again for 20 days after the period of experimental treatment. During that time the newly formed initial buds in angles of needles of decapitated plants were not removed and they grew out in the new shoots. Resting-buds at the top of lateral shoots in plants that had been submitted to short photoperiods (group *b*) began to develop but the buds at top of main shoot of these plants as well as those only with the top of main shoot covered (group *c* and group *d*) did not develop within that time.

Anatomical examination showed that only plants subjected to short photoperiods formed thick-walled tracheids differing significantly from cell-wall thickness of all other groups of plants (Table 1). Neither the

Table 1

Cell wall thickness of tracheids formed under various experimental treatment
(in microns)

Experimental treatment	Wood formed at the be- ginning of experiment	Wood formed under vario- us experi- mental treatment	$\mu \cdot t_x$
a) Continuous illumination	2.8	3.6	0.4
b) Short day	2.7	5.6	0.4
c) Continuous illumination, only the top of main shoot covered	2.8	3.4	0.3
d) Continuous illumination, the top of main shoot covered and lateral shoots decapitated	2.8	3.3	0.5
e) Continuous illumination, lateral shoots decapitated	2.6	3.4	0.4
$\mu \cdot t_x$	Fe 0.77 F _t 3.44	0.4—0.5	

Statistical computations by Snedecor's method, t-test at 1 percent level.

Table 2

Radial diameter of tracheids formed under various experimental treatment
(in microns)

Experimental treatment	Wood formed at the be- ginning of experiment	Wood formed under vario- us experi- mental tre- atment
a) Continuous illumination	11.6	13.3
b) Short day	12.0	13.0
c) Continuous illumination, only the top of main shoot covered	11.4	12.7
d) Continuous illumination, the top of main shoot covered and lateral shoots decapitated	11.5	11.8
e) Continuous illumination, the lateral shoots decapitated	12.0	13.8
$\mu \cdot t$	1.4—1.6	

covering of the top of main shoot, nor decapitation of lateral shoots did induce the formation of thick-walled tracheids. The differences between cell-wall thickness of tracheids of plants in the above conditions and of intact plants throughout under continuous illumination were not significant.

The cell-wall thickness of tracheids formed at the beginning of experiment in those groups (c, d, e) of plants was smaller than that of last formed but the differences were of the same significance as in intact control plants under continuous illumination (group a).

The examination of wood showed the absence of essential differences between the mean radial diameter of tracheids in all groups of plants (Table 2).

The radial diameter of tracheids formed both at the beginning of experiment and under various experimental treatments corresponded to the radial diameter of tracheids of late wood in natural conditions, (Table 3).

Table 3

Cell wall thickness and radial diameter of early and late wood tracheids of plants grown under natural conditions
(in microns)

	Early wood	Late wood	$\mu . t$
Cell-wall thickness	3.0	6.9	0.4
Radial diameter	18.5	12.6	1.5

In the earlier experiments with larch (Wodzicki 1961a), however, it was showed that the decapitation, if only of the lateral shoots, caused the decrease of radial diameter of tracheids. This reduction of radial diameter was not associated with any increase of thickness of cell walls of tracheids. For this reason, an additional experiment was made. Three spruce plants, similar to those described above, all the time subjected to continuous light conditions, were completely decapitated. Newly formed initial buds on the shoots of these plants were being removed during the following 20 days. After this period, the appearing buds were allowed to grow again for the next 30 days and they formed new shoots.

The anatomical examination of wood revealed that in all cases, a few layers of tracheids with greatly reduced radial diameter were formed as compared with analogical tracheids of intact plants under continuous illumination (Fig. 1). In this region of wood, it was observed, that 1 to 3 layers of tracheids preserved their protoplasmatic content and the cell

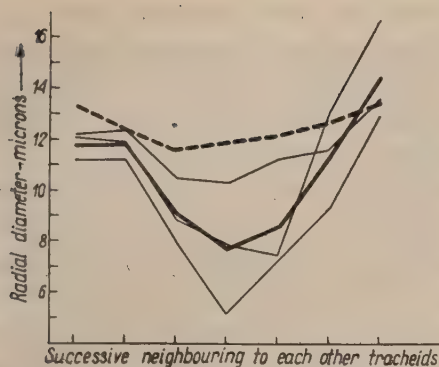


Fig. 1. Effect of complete decapitation of plants on the radial diameter of tracheids.

Continuous lines — successive tracheids in the zone of reduced radial diameter in decapitated plants; dotted line — tracheids in intact plants; heavy lines — denote the mean values of three plants; light lines — the mean values of individual plants

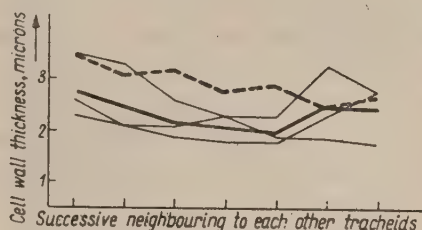


Fig. 2. Effect of complete decapitation of plants on the cell-wall thickness of tracheids.

Continuous lines — successive tracheids in the zone of reduced radial diameter in decapitated plants; dotted line — tracheids in intact plants; heavy lines — denote the mean values of three plants; light lines — the mean values of individual plants

walls of tracheids was somewhat thinner than in intact plants (Fig. 2 and Fig. 3 c,d). The walls of the former were also less stained with safranin, probably owing to the lower degree of lignification.

DISCUSSION

The experiments confirmed the results of previous investigation on the one-year old larch plants. It was demonstrated that the shoot apex of the main shoot may be induced to dormancy with short photoperiods exerting an effect on the young growing needles or the shoot apex itself. Besides, the rest-inducing stimulus arising under short-day treatment was not transferred to other shoots growing under continuous illumination. Thus, the results provide an additional evidence that the individual shoots may be largely independent from each other in respect of extension growth and onset of dormancy. It is noteworthy, that the photoperiodic reaction of shoot apex occurred in spruce, as in the earlier experiments with larch, although in each case there were different light and especially different temperature conditions. The experiments with larch were conducted under incandescent light in greenhouse where the diurnal amplitude of temperatures was considerable. As in the case of larch, it was demonstrated that short photoperiods caused the formation of thick-walled tracheids. Neither the cessation of height growth (as

result of placing the top of main shoot under cover combined with removing of all other growing points in plant) nor the temporary complete decapitation did induce the thick-walled tracheids formation. Therefore, it may be assumed that also in spruce, the process of cell-wall thickening of tracheids was not directly related to the termination of the extension growth of shoots. The experiments provide an indirect evidence that most important for the process bringing about cell-wall thickening was the type of photoperiodic treatment to which full grown needles were submitted. Nevertheless, it cannot be discounted that a few layers of tracheids in the wood of plants completely decapitated were not fully differentiated (the tracheids which preserved the protoplasm). The walls of these cells were somewhat thinner and probably the degree of their lignification was lower than those of the control plants. A similar response to decapitation was also observed earlier in larch seedlings (Wodzicki unpublished data) and in the study of wood formed after ring barking in other woody species (literature recently reviewed by Wardrop, Bland 1959). These disturbances of the normal wood differentiation may also, to some extent, be compared to the formation of large resin ducts after removing a strip of bark and phloem round the stem of 1-year old larch (Wodzicki 1961b) and probably should be regarded as connected with the mechanical damage rather than with the extension growth inhibition.

This problem requires, however, a further study, especially taking into account the results of experiments in which the influence of growth substances on differentiation and lignification of conducting elements of wood was evident (Jacobs 1952, Wareing 1958 and others).

The radial diameter of tracheids formed both at the beginning of experiment and under the various experimental conditions (except for complete decapitation) corresponds to the radial diameter of tracheids of late wood. This means, that the radial diameter of tracheids formed under continuous illumination was smaller than the radial diameter of early wood tracheids.

Probably, this can be partially explained by taking into account the fact that the experimental plants were transplanted into pots shortly before they were transferred to continuous illumination, while the plants in natural conditions had been growing for two years at the same site.

Another possibility which does not exclude the first, is that the process of radial diameter growth of cells in cambial zone was more affected by the provided temperature conditions which were found less favourable for growth than the periodic changes of temperature during the day (Kramer 1958).

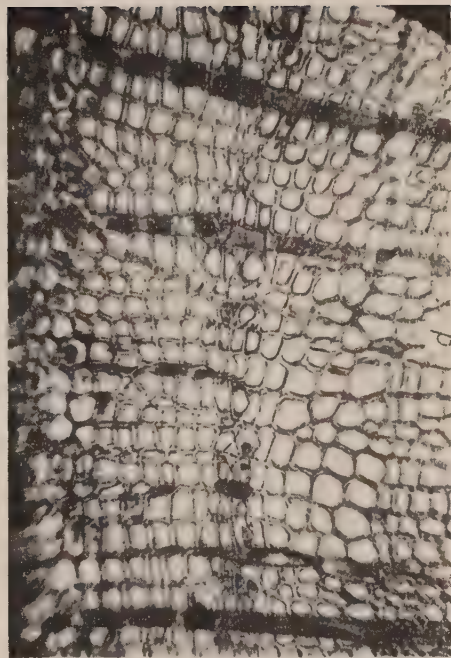
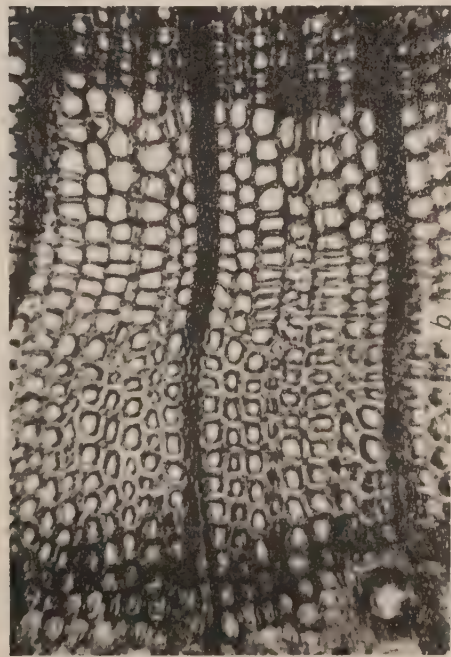
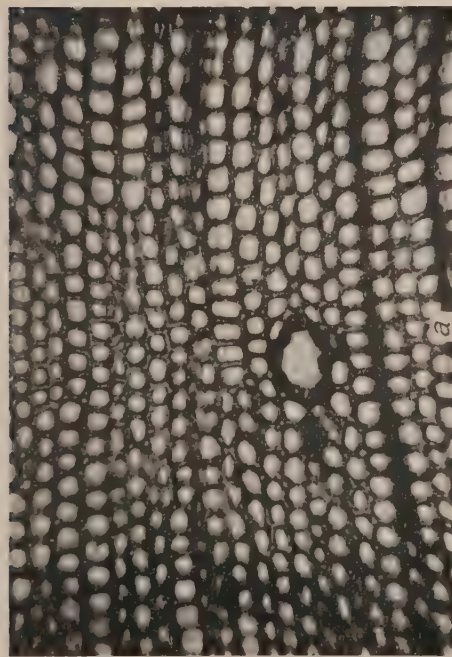


Fig. 3. *a* — Wood formed under continuous illumination; intact plants, *b* — Wood formed under short day conditions; intact plants, *c*, *d* — Wood formed under continuous illumination; decapitated plants

Although, the question cannot be sufficiently clarified on the basis of the present investigation the experiment with completely decapitated plants showed that the process of radial diameter growth of tracheids may be suppressed by the removal of all growing apices. It may be inferred from this observation that there is a dependence of radial diameter growth of tracheids upon the extension growth of shoots as suggested in the earlier experiments with larch.

SUMMARY

Some aspects of the influence of short-day conditions on the height growth and wood formation of 3-year old spruce (*Picea abies* (L.) Karst.) plants were studied. Whole plants or only the top of main shoot were subjected to 12-hours photoperiods. It was shown, as in earlier experiments with larch, that the apex of main shoot may be induced to dormancy separately by placing under cover the apical part of shoot only, during extension growth of all the lateral shoots. Therefore, the type of photoperiodic conditions to which young growing needles or the growth apex itself were submitted was the most important factor affecting extension growth regardless of the photoperiodic treatment to which were subjected other aerial parts of the plant.

The anatomical examination of wood showed that only when the whole plant was influenced by short-day conditions the thick-walled tracheids were formed. Neither the covering of the top of main shoot (even when all lateral shoots were decapitated) nor the full decapitation induced thick-walled tracheids formation. The decapitation of all growing shoots in plants under continuous illumination, however, caused the reduction of radial diameter of tracheids and disturbed their normal differentiation. The results are discussed.

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Sweet-Water Algae of Antarctica

O. S. VIALOV and N. W. SDOBNIKOVA

The plant kingdom of Antarctica is almost entirely composed of lichens, mosses, and algae. To the several collections of Antarctic plants a new one composed mainly of land algae was added by O. S. Vialov in 1956.

The algae in Vialov's collection were identified by N. W. Sdobnikova at the Botanical Institute of the Academy of Sciences of the USSR. This paper describes briefly the identified specimens and the stands where they were collected formulating at the same time some conclusions on the occurrence of this algae.

The sweet-water algae of Antarctica are very little known. In the 19th century and at the very beginning of this century a few reports were published describing the algae from the coasts of Victoria Land, of Graham Land, and from some neighbouring islands. All the data then available were assembled by N. Wille in 1924 in his report on the sweet-water algae collected by the German expedition on the ship "Gauss" of 1901 to 1903 led by E. Drygalski. The German expedition explored the same territories as the 1956 Antarctic Expedition of the Academy of Sciences of the USSR. From this area N. Wille reported 16 species.

In the other parts of the Antarctic coast between 98° and 107° East longitude explored by the Soviet expedition in 1956 (Vialov 1956) sweet-water algae were unknown.

Among the specimens of this investigation 27 species and forms were identified, six of which were previously unknown on Gauss Mt. and Victoria Land. A characteristic trait of this flora is its specific variability. Only very few species of those brought by the Soviet expedition occur also on the lists compiled by various explorers for other parts of Antarctica. The only species recurring on almost all the lists is *Prasiola crispa* Menegh.

O. S. Vialov's collection was assembled on Gauss Mt. in the neighbourhood of the Mirnyj Station (Mirnyj I and Folmar) and on Bunge's Oasis where they were thrown out on the shore of Figurnoje Lake and of another small unnamed lake.

Here is a short description of these stands.

1. Gauss Mt., the easternmost point visited by our expedition, is a volcanic cone rising 371 m. above sea level. It is built of basaltic lava probably of Upper Tertiary origin. On the north side the mountain drops by a cliff into the sea and to the south there is an ice-field extending at its foot. The south strongly eroded slope of the cone is covered with fine basaltic rubble. At the bottom of this slope at the altitude of a few metres a small stand of half dried algae was found on February 21 among the rock fragments. From this stand two species were identified: *Phormidium tenue* (Menegh.) Gom. and *Chlorella vulgaris* Beyerinck. The second of these species is not on the list of the German expedition of 1901—1903, though the species in that collection are far more numerous (Wille 1924).

2. Along the coast in the vicinity of Mirnyj Station there are black hillocks built of granite and gneisses. The South-East slope of the highest hillock in Mirnyj I has been named the Cape of Ivan Khmara in memory of the tractor-driver killed in an accident in 1956. On this slope specimens of algae were collected on January 21.

Adjoining to the main snow-covered slope there is a flat rock platform where water from the melting snow flows down the rocks in tiny rivulets forming numerous small pools sometimes connected by minute waterfalls and water-filled crevices. The pools are up to one metre long and about 10 cm deep. The bottom of the pools is covered with sand, small unrounded stones, and penguin feathers left over from the sheddings of the previous seasons. In the main chain of the pools with flowing water the stones and the feathers, at the bottom as well as a parts of the banks were covered by a very thin brown film with small bubbles of air caught under it. The film was mainly composed of blue-green algae, mostly from the genus *Phormidium*. In one place on a stretch of sand washed over by flowing water there were a few green tufts of green algae which looked like "salad leaves".

The full list of species from this stand is as follows: *Synechococcus major* Schröt. f. *crassus* (Archer) Elenk., *Microcystis muscicola* (Menegh.) Elenk., *M. parietina* (Näg.) Elenk., *M. pulvereae* (Wood) Forti emend. Elenk. f. *irregularis* (B. Peters) Elenk., *Gloeocapsa crepidinum* Thur., *Chlorogloea* sp., *Stratonostoc* Linckia (Roth.) Elenk., *Phormidium autumnale* (Ag.) Gem., *Ph. frigidum* F. E. Fritsch, *Ph. subcapitatum* B. Peters., *Schizothrix Lenormandiana* Gom., *Chlorococcum infusionum* (Schränk) Menegh., *Chlorella vulgaris* Beyerinck, *Prasiola crispa* Menegh., *Tabellaria flocculosa* (Roth) Kutz., *Navicula* sp., *Hantzschia amphioxys* (Ehr.) Grun.

Prasiola crispa and various *Phormidium* species were the most numerous, whereas the other algae were only an admixture.

3. On Folmar Island, the closest island off Mirnyj Station, algae were collected on January 15 in the central part of the southern coast of the island. The island is built of substratum rocks (granitoids). The algae were collected in a small crevice in which sand and gravel from the weathering of rocks saturated with water from the melting snow accumulated.

On this stand the algae formed a bright green patch. The growth habit of the algae was of two clearly distinguishable kinds: 1) there were tufts of minute "salad-like" leaves 2—3 mm. high (*Prasiola crispa*) and 2) patches of round, merging clods composed of tiny globules like pin-heads.

The species found on this stand were: *Microcystis muscicola* (Menegh.) Elenk., *M. pulvereae* (Wood) Forti emend. Elenk., *M. pulvereae* (Wood) Forti emend. Elenk. f. *irregularis* (B. Pe-

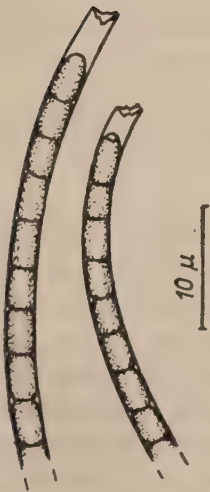


Fig. 1. *Phormidium subcapitatum* B. Peters

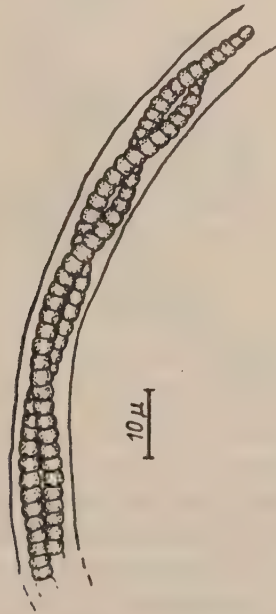


Fig. 2. *Schizothrix Lenormandiana* Gem.

ters) Elenk., *Gloeocapsa crepidinum* Thur., *Phormidium autumnale* (Ag.) Gom., *Ph. tenuissimum* Woronich., *Chlorococcum infusionum* (Schrank) Menegh., *Chlorella vulgaris* Beyerinck, *Prasiola crispa* Menegh.

At the end of January O. S. Vialov and some other members of the expedition spent several days in the Bunger Oasis, a rather large tract of land with no ice cover. The area there is strewn with small hillocks built of crystalline rocks: granites, gneisses, and crystalline shales.

The valleys between the hillocks are filled with morain material and at their bottom there often are small lakes, some with sweet water others with brackish or even salt water. The camp of the expedition was pitched on the shore of the largest lake which has been named Figurnoje Lake. The Polish "Dobrowolski" Research Station is now situated near this lake.

4. The Figurnoje Lake has flowing water, it is up to 300 m. wide and is very long. It fills a deep winding valley with several bands and side branches making its shape very complicated.

The northernmost part of the lake to the West-North-West of the camp has a distinct character. At this end the lake discharges into a torrent connecting the Figurnoje Lake with another lake which has been named Lagiernoje Lake. The flat and stony northern shore of the

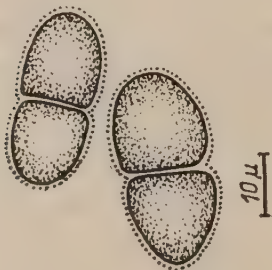


Fig. 3. *Synechococcus major* Schröt.
f. *crassus* (Archer) Elenk.

Lagiernoje Lake extends to the precipitous valley side. On this flat shore about 1.5 km. West of the camp a patch of algae thrown out from the water was found on January 29. They were mainly blue-green filamentous algae.

The following species were identified from this stand: *Microcystis muscicola* (M en e g.) E len k., *Amorphonostoc paludosum* (K ü t z.) E len k., *Phormidium Retzii* (A g.) G om., *Plectonema Battersii* G om., *Chlorella vulgaris* B e y e r i n c k.

5. Another small lake about 150 m. in diameter was found about one kilometre North-West of the camp in the Oasis. The lake is easy to find because it lies at the prolongation of an elongated surface outcrop of black gabbro-dabase stretching north-westward from the camp. There is another outcrop of gabbro-dabase at the border of a small valley with a salt-water lake at the bottom. Along the gently sloping shore of this lake was a band of algae up to 1.5 m. wide and sometimes even wider. On

this stand the dominant species were blue-green filamentous algae and diatoms (*Amphora* sp.).

The list of species collected from this stand on January 25 is as follows: *Gloeocapsa crepidinum* Thur., *Phormidium Bohneri* Schmidle, *Ph. favosum* (Bory) Gom., *Ph. tenuissimum* Woronich., *Plectonema Battersii* Gom., *Pl. Boryanum* Gom., *Chlorococcum infusionum* (Schrank) Menegh., *Chlorella vulgaris* Beyerinck, *Amphora* sp.

All the identified algae are of three types: *Cyanophyta* (blue-green algae), *Chlorophyta* (green algae), and *Bacillariophyta* (diatoms). The various species of blue-green algae are the most numerous on the list:

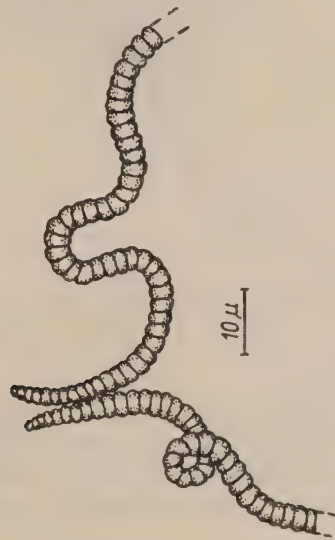


Fig. 4. *Plectonema Battersii* Gom.

they include 20 species which makes 74 per cent of the total number of species. The order *Cyanophyta* is represented by four suborders, the most important of which is the suborder *Oscillatoriales*. We have here a special instance of the general law according to which the representatives of a suborder having the greatest adaptability are the first to occupy the habitats with the most unfavourable conditions. E.g. on the mineral substratum of clay deserts the only pioneers of the plant kingdom are the representatives of the suborder *Oscillatoriales* covering large stretches with a thin felt-like film. On sand and clay deserts, on the barren rocks of polar regions, in hot springs, and in all places where the conditions are extremely unfavourable for the development of life the first place with regard to the number of species and expansion is always occupied by the blue-green algae from the suborder *Oscillatoriales*.

The majority of the *Cyanophyta* species covered by this investigation have a wide ecological amplitude. Only a few of these species are usually found in high latitudes where they have their habitats in cold waters or on cold soils: they are *Synechococcus major* f. *crassus* (fig. 3), *Phor-*

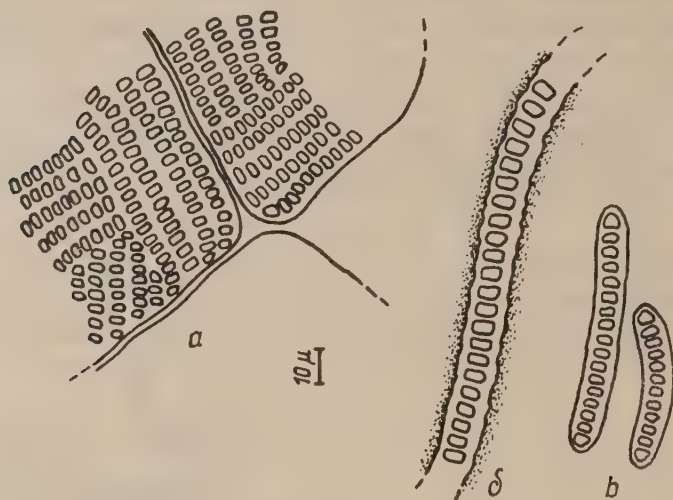


Fig. 5. *Prasiola crispa* Menegh., a in the form of membranes, b — in the form of long and short threads

midium tenuissimum, *Ph. subcapitatum*, and *Ph. frigidum*. The typically marine species are *Plectonema Battersii* (fig. 4) and *Gloeocapsa crepidinum*. Worth stressing is the presence in the collection of the rare species *Schizothrix Lenormandiana* (fig. 2) first found on the French coast.

Among the green algae only three species belong to the order *Chlorophyta*. Two representatives of this order belong to the suborder *Proto-*

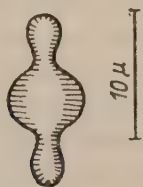


Fig. 6. *Tabellaria flocculosa* (Roth) Kütz.

coccales i.e. *Chlorella vulgaris* and *Chlorococcum infusionum*. Both these species have a very wide distribution extending from the highest to the lowest latitudes. The third species of this order, *Prasiola crispa* from the

suborder *Ulothrichales* (fig. 5 a, b, d), has a rather wide distribution in the world and in Antarctica. A well known ecological characteristic of this last species is its very strong nitrophily. In places where birds collect (in this case penguins) and where the soil is covered with large amounts of their droppings *Prasiola crispa* forms large, silky, wrinkled patches looking like a turf cover clearly discernable with the naked eye.

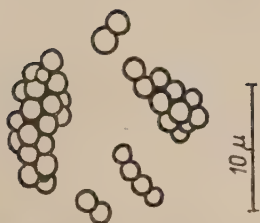


Fig. 7. *Microcystis pulverea* (Wood) Forti emend. Elenk.

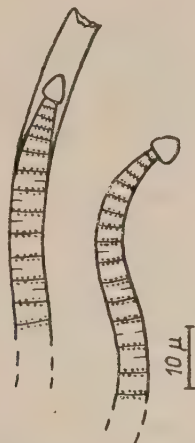


Fig. 8. *Phormidium favosum* (Bory) Gom.

The shed penguin feathers are covered by a thin green-brown film usually formed by two species: *Prasiola crispa* and *Phormidium subcapitatum*. This last species has so far been reported only from Iceland and probably also is a nitrophilous species.

There are only a few species of diatoms in the list of this collection the most common among them being *Hantzschia amphioxys*. As is known this species is almost ubiquitous and has an exceptional ability for adaptation to extremely different conditions. *Tabellaria flocculosa* (fig. 6), which is another representative of the *Bacillariophyta*, is better known as an inhabitant of the northern latitudes and of mountainous regions, but also is an eurythermous species.

A genetically and ecologically interesting trait of the algae of Antarctica is that the dried algae placed in a liquid nutrient medium in June of 1956 did not begin to show signs of life till November, i.e. at the time when the Antarctic summer begins.

The methods applied for the investigation of land algae of moderate latitudes may be inadequate for investigations on Antarctic algae. For

this reason this report on the algae flora from the stands round Mirnyj Station cannot be considered as final and complete.

The list of the collected and identified species of algae is as follows:

CYANOPHYTA

Family Coccobactraceae

1. *Synechococcus major* Schröt. f. *crassus* (Archer) Elenk. (fig. 3)

Family Microcystidaceae

2. *Microcystis muscicola* (Menegh.) Elenk.
3. *M. parietina* (Näg.) Elenk.
4. *M. pulverea* (Wood) Forti emend. Elenk. (fig. 7)
5. *M. pulverea* (Wood) Forti emend. Elenk. f. *irregularis* (B. Peters.) Elenk.

Family Gloeocapsaceae

6. *Gloeocapsa crepidinum* Thur.
7. *Chlorogloea* sp.

Family Nostocaceae

8. *Amorphonostoc paludosum* (Kütz.) Elenk.
9. *Stratonostoc Linckia* (Roth) Elenk.

Family Oscillatoriaceae

10. *Phormidium autumnale* (Ag.) Gom.
11. *Ph. Bohneri* Schmidle
12. *Ph. favosum* (Bory) Gom. (fig. 8)
13. *Ph. frigidum* F. E. Fritsch.
14. *Ph. Retzii* (Ag.) Gom.
15. *Ph. subcapitatum* B. Peters (fig. 1)
16. *Ph. tenue* (Menegh.) Gom.
17. *Ph. tenuissimum* Woronich

Family Schizothrichaceae

18. *Schizothrix Lenormandiana* Gom. (fig. 2)

Family Plectonemataceae

19. *Plectonema Battersii* Gom. (fig. 4)
20. *P. Boryanum* Gom.

CHLOROPHYTA

Family Protococcaceae

21. *Chlorococcum infusionum* (Schrank) Menegh.
22. *Chlorella vulgaris* Beyerinck

Family Blastosporaceae

23. *Prasiola crispa* Menegh. (fig. 5a—B)

BACILLARIOPHYTA

Family Tabellariaceae

24. *Tabellaria flocculosa* (Roth.) Kütz. (fig. 6)

Family *Naviculaceae*25. *Navicula* sp.26. *Amphora* sp.Family *Nitzschiaceae*27. *Hantzschia amphioxys* (Ehr.) Grun.

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A Comparative study on *Dactylis slovenica* Dom. and *D. glomerata* L.

A. DOROSZEWSKA

The great morphological variability in the genus *Dactylis* makes the study of the systematics of this economically important genus of grasses very difficult. In most cases the variability is continuous and there are no traits clearly distinguishing the particular forms. Neither are there any sterility barriers so that the various formes can cross with each other giving fertile hybrids. The lack of clearly marked interspecific differences has been the cause of much inconsistency in the systematic treatment of this genus by various workers. The *Dactylis* forms which have been discriminated are defined by some florists as separate species and by others merely as subspecies. Cytological investigations have revealed two chromosome levels within the genus *Dactylis*: one diploid with $2n=14$ and the other tetraploid with $2n=28$ (Levan 1930, Katterman 1931, Müntzing 1933 and 1937). In the course of an investigation on *Dactylis* Stebbins and Zahory (1959) reached the conclusion that the genus consists of one common species *Dactylis glomerata* L. in which two groups are discernable, one diploid the other tetraploid. They studied in detail the group of 11 diploid subspecies growing mainly in the Mediterranean area and in South Europe.

The tetraploid forms of *Dactylis* spreading in Europe and Asia from the polar to the subtropical regions have a much wider distribution than the diploids. The tetraploids are a very numerous and variable group of plants which as yet is little known, though the first steps towards a detailed investigation have already been made. In 1961 Borrill distinguished the new tetraploid species *Dactylis marina* Borrill occurring along the Atlantic and Mediterranean coasts of Europe. The present investigation on *D. slovenica* may also add some details to the knowledge of the tetraploid forms of *Dactylis*.

Dactylis slovenica was described by Domin in 1923 (Domin 1929) as a new species occurring in the Carpathians on lime and dolomite substrata. At that time four main forms of *Dactylis* with distinctly different distributional areas were distinguished in Europe.

These forms were *Dactylis glomerata* L., *D. Aschersoniana* Graebn.,

D. hispanica Roth., and *D. slovenica* Dom. In 1943 Domin published a monography of the genus *Dactylis* in which he defined *D. slovenica* and all other above-mentioned forms as subspecies of *D. glomerata* L. In this way *D. slovenica* was degraded by its discoverer to the rank of a subspecies, but treated on equal terms with *D. Ascher-soniana* and *D. hispanica*.

Domin discovered *D. slovenica* for the first time in the Belanske Tatry massif, which forms part of the Slovenian Tatras, whence the name *D. slovenica*. In further observations Domin found that *D. slovenica* was a montane plant growing in the Carpathian Mts. to the East Carpathian massif in the East and to the Karkonosze and Moravia in the West. In this area it occurs together with *D. glomerata*.

On the slopes of the Belanske Tatry massif *D. slovenica* grows in dense high tufts together with such species as *Cimicifuga foetida*, *Aruncus silvester*, *Aconitum firmum*, *Digitalis ambigua*, *Senecio Fuchsii*, *Mulgedium alpinum* etc. The plants of *D. slovenica* are conspicuous by their vigorous growth habit. Their widely outspread and drooping panicles differ so much from the appearance of *D. glomerata* that at first sight they seem to constitute a very distant form. However, on closer examination the differences between *D. glomerata* and *D. slovenica* are seen to be less clearly marked and, therefore, the question arises whether *D. slovenica* is in fact a separate species, a subspecies or only one of the numerous modifications of *D. glomerata*. The investigation of the differences and the similarities between the two forms has been the aim of this work.

MATERIAL AND METHODS

The examinations of *D. slovenica* and *D. glomerata* were carried out on plants growing in their natural habitats and on plants transferred from their natural stands and cultivated in the Botanical Garden of the Warsaw University. The plants described by Domin as *D. slovenica* grew in the Belanske Tatry massif in the valley of the stream Regliany Potok near a locality called Siroke Pole. The altitude of the valley was about 1250 m. above sea level and the soil limy. The plants for this investigation were collected in 1956 in the Belanske Tatry massif in the Holubyho Dolina valley the altitude of which was approximately the same as of the Regliany Potok valley. In his list of stands of *D. slovenica* in the Tatras Domin in 1943 also mentioned the Holubyho Dolina valley. Somewhat below the mountain shelter in this valley there is a small dell called the Dolina Sedmi Promenov where there are stands of typical *D. slovenica*. The living specimens of *D. slovenica* for this investigation were collected from four stands situated not far from each other in Holubyho Dolina valley. The first of these stands was situated near the path running just

above the Sedmi Promenov waterfall at the altitude of 1350 m, above sea level. On this stand besides the typical *D. slovenica* specimens there were also *D. glomerata* plants and natural hybrids between these two forms. The second stand was in the Dolina Sedmi Promenov dell near the waterfall at the altitude of 1275 m. This stand was the dampest of all and *D. slovenica* plants growing there were very vigorous and morphologically uniform. The third stand was situated at the bottom of the Dolina Sedmi Promenov dell at the altitude of 1225 m. Here, similarly as on the first stand, *D. slovenica* was growing together with *D. glomerata* and their hybrids. The fourth stand was situated in the Holubyho Dolina valley above the Dolina Sedmi Promenov dell at the altitude 1370 m., on the south-east slope cleared of the forest which had once grown there. This stand was the driest of the four stands described here: the plants growing there abundantly were morphologically uniform.

The *D. glomerata* plants used as the comparative material for this investigation were collected from various lowland and mountain stands in Poland.

The list of these plants cultivated in our Botanical Garden used for the investigation was as follows:

D. slovenica:

- no. 55 from stand 1 in the Tatras,
- no. 56 to 60 from stand 2 in the Tatras,
- no. 49 from stand 3 in the Tatras,
- no. 43 to 45 from stand 4 in the Tatras,
- no. 1 from Dolina Sedmi Promenov dell in the Tatras,

Natural hybrids *D. slovenica* × *D. glomerata*:

- no. 52, 53, 54 from stand 1 in the Tatras,
- no. 46, 47, 50 from stand 3 in the Tatras,

D. glomerata from mountains stands:

- no. 51, stand 1 in the Tatras,
- no. 49, stand 3 in the Tatras,
- no. 63, 64, 65, a rocky stand sparsely covered with mountain pine in the Morskie Oko valley in the Tatras,
- no. 21 to 30, a meadow near the Droga Pod Reglami road in Kiry near Zakopane,
- no. 31 to 40, a meadow near the stream in Bukowina Tatrzńska Dolna,

D. glomerata from lowland stands:

- no. 4, 5, 6, 7, open roadside stands in Salomea near Warsaw,
- no. 11 to 20, stands in a this mixed forest in Zalesie near Warsaw,
- no. 41, 42, 61, 62, the Puszcza Białowieska forest,
- no. 2, 3, seaside, thinly forested sand dunes in Leba on the Baltic Sea coast.

In the Botanical Garden all the *Dactylis* plants were grown side by side under the same conditions, on clayey-soil beds with strong insolation. The plants were grown from 1956 to 1960. Every year they were measured, their blooming seasons were recorded, and they were examined with regard to their vigour, pollen fertility etc.

The crosses were made by the methods of castration and of additional pollination.

The material for cytological examinations was fixed in 1:3 aceto-alcohol and stained in propiocrarmine. Pollen fertility counts were made on smears stained in 1:1 acetocarmine with glycerine.

THE MORPHOLOGY OF *D. SLOVENICA* AND *D. GLOMERATA*

Dactylis slovenica is a montane species of the Carpathians occurring most abundantly on lime substrata in the Tatras. It grows in thin forests, on pastures, and on meadows reaching from the sub-Alpine to the sub-mountain stage. In its mountain distribution *D. slovenica* meets with *D. glomerata*, the latter species being able to grow at considerable alti-



Fig. 1. Schematic diagrams showing the growth habits of *Dactylis glomerata* tufts: 1 — culms almost straight, leaves growing almost vertically with only the tips drooping; 2 — culms slightly bent in the nodes, leaves of the same kind as in 1; 3 — culms same as in 2, leaves bending outward; 4 — culms strongly arched, leaves bending outward

tudes in spite of its lowland origin. The highest altitude at which Kotula (1889—90) found *D. glomerata* was 1707 m. near Zimne Źródła in the Tatras. In the Alps *D. glomerata* was found at the altitude of 2320 m. (Hegi 1907). A characteristic trait of *D. glomerata* is its great morphological variability and, therefore, to establish the systematic position of *D. slovenica* the morphology of the latter is here compared to the characteristic traits of the former. For this purpose many plants of *D. glomerata* coming from various parts of Poland were examined but in the compara-

tive tables only the most typical individuals from the particular stands are described.

Growth habit. The plants of *D. slovenica* are vigorous, the culms are strong, thick, and slightly bent at the nodes so that the flower culms are arched.

In the case of *D. glomerata* four main types of the growth habit have been discriminated in the course of comparative examinations. The schematic diagrams of these four types are shown in fig. 1. The growth habit of the tufts, as shown by these diagrams, depends on the magnitude of the bend in the nodes and on the position of the basal leaves which are either almost vertically upright with only the ends drooping or curved towards the ground. Between the four main types there are continuous series of intermediate forms.

The distribution of the growth habit types in *D. glomerata* and *D. slovenica* is shown in Table 1. As is to be seen the variation of the growth habit of *D. slovenica* ranges between types 3 and 4 and is not as wide as the variation of *D. glomerata*.

Table 1

The types of the growth habit of tufts according to fig. 1

No. of plants	The types of the growth habit			
	1	2	3	4
<i>D. slovenica</i>			44 56 57 58 59	1 43 45 48 55
<i>D. glomerata</i>				
from lowland stands	2 3 4 5 6	61 62	18	41 42
from mountain stands	51	31 32 33 36 37 40 63 64 65 49	34 35 38 39	

The size of culms and leaves. The plants of *D. slovenica* are somewhat larger than the plants of *D. glomerata*, but the difference is not significant. The height of the culms in *D. slovenica* is about 1.5 m., whereas among the *D. glomerata* plants there are much lower individuals, some of them less than one metre tall, e.g. plants no. 41 and 42 from the Puszcza Białowieska forest, and individuals of the same size as *D. slovenica*, e.g. plants no. 2 and 3 from Leba. The basal leaves of *D. slovenica* are about one metre long and their widths range 7 to 13 mm., whereas the widths of the leaves on the culms range up to 15 mm. In the most vigorous specimens of *D. glomerata* the length of the basal leaves is not

more than 80 cm. and the widths of these leaves range 4 to 12 mm. This means that the basal leaves in *D. slovenica* are somewhat larger than in *D. glomerata*. However, in *D. slovenica* plants grown in the Botanical Garden in Warsaw the length of leaves gradually decreased and after three years of cultivation it was of the same order as in *D. glomerata*. On the other hand, the height of the culms did not decrease and after five years of cultivation the maximum height of the culms in *D. slovenica* was 1.91 m.

The length of the ligule ranges 3 to 8 mm. in *D. glomerata* and 4 to 7 mm. in *D. slovenica*.

The colour of plants. The plants of *D. slovenica* are usually bright green similarly as many specimens of *D. glomerata* from both mountain and lowland stands. In *D. glomerata* the colour of plants ranges from pale green — e.g. specimens no. 61 and 62 from the Puszcza Białowieska forest — through bright green, to bluish-green and even grayish green as in the plants no. 2 and 3 from Łeba. (Table 2).

Table 2
The colour of plants

No. of plants	Pale green	Bright green	Bluish green	Grayish green
<i>D. slovenica</i>	57 60	1 43 44 45 55 56 58 59	48	
<i>D. glomerata</i>				
from lowland stands	61 62	41 42	20 29	2 3
from mountain stands	31	32 33 40 63, 64 65	34 35 36 37 38 39 49 51	

The stiffness and the harshness of plants. The stiffer plants usually are also harsher, since both these traits depend on the content of silica. Lackamp (1955) demonstrated that the *D. glomerata* plants containing more silica had more sharp toothlets on the margins and the ribs of leaves and were thus harsher to the touch.

Among the plants of *D. glomerata* four degrees of harshness have been discriminated: smooth, slightly harsh, harsh, and very harsh.

The plants of *D. slovenica* are smooth or slightly harsh (Table 3).

The swelling of the culm base. The base of culms in *D. slovenica* is distinctly bulbed. The swelling is caused by a thick reduced stem inside the base of the culm. Being hard and lignified these reduced stems act in a way a reinforcement of the culm bases, thus improving the attach-

ment of the plants to the substratum. According to Domin (1929, 1943) the swelling at the base of culms is the main morphological trait distinguishing *D. slovenica* from the other *Dactylis* species.

The bulb-like swelling at the culm bases in *D. slovenica* is well developed in old culms growing separately outside the tufts. The young

Table 3

The harshness of plants

No. of plants	Smooth	Slightly harsh	Harsh	Very harsh
<i>D. slovenica</i>	1 43 44 45 57 60	48 55 56 59		
<i>D. glomerata</i>				
from lowland stands	61 62	41 42	18	2 3
from mountain stands	31 32 33 34 35 56 38 40 63 64 65	37	49 51	39

culms and the culms growing inside the compact tufts are not bulbed. The reason for this may simply be the mechanical pressure of the neighbouring tightly packed culms which do not allow the swelling to develop.

The swellings on the culms of *D. slovenica* are up to 2 cm. long and about 1.2 cm in diameter.

The plants of *D. glomerata* also have such a lignified culm, but it is much smaller than in *D. slovenica*. Its diameter is not more than 0.5 cm. and, therefore, the culm base is usually even or only slightly swollen.

In connection with the presence of the swelling on the culm base in *D. glomerata*, though much smaller than in *D. slovenica*, the question had to be considered whether the difference between the two forms in the development of this trait was not caused by the greater thickness of the whole culms in *D. slovenica*. In order to obtain quantitative data on this trait the thickness of the culms and of the swellings on them was measured. Accepting the diameter of the culm directly above the swelling as the independent variable X and the diameter of the swelling as the dependent variable Y, the regression coefficient for both forms was calculated. The ten thickest culms were measured in ten individuals of *D. slovenica* and in ten of *D. glomerata*.

The calculated regression coefficient was $b=1.88$ for *D. slovenica* and $b=1.19$ for *D. glomerata*. The differences between these coefficients are represented graphically in fig. 2.

The measurements and the calculated regression coefficients show that the swelling of the culm bases is much more developed in *D. slovenica* than in *D. glomerata* and that this trait does not depend on the difference in the thickness of the culms. The swelling of the culm base is certainly one of the most characteristic traits of *D. slovenica*.

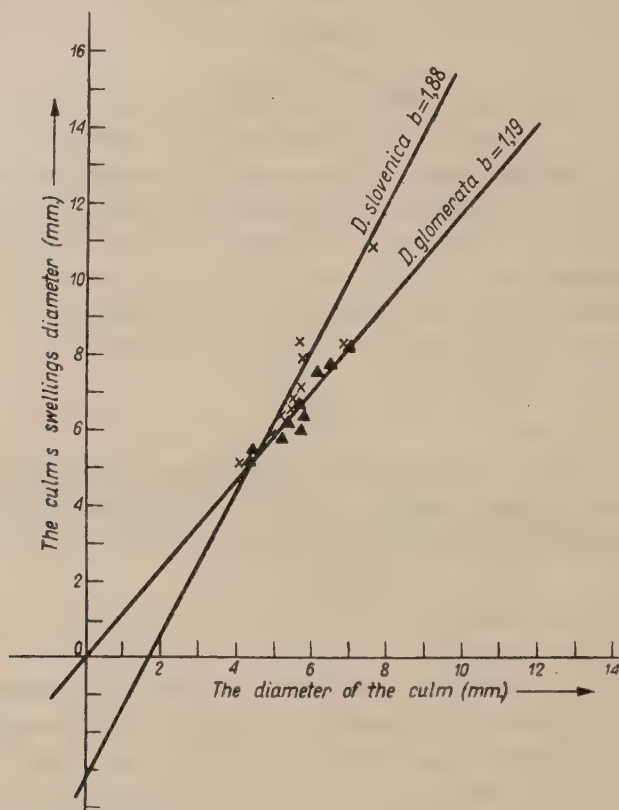


Fig. 2. Graph showing the regression of bulb diameter to culm diameter for *D. slovenica* and *D. glomerata*

The panicle. The differences in the structure of the panicles between *D. slovenica* and *D. glomerata* are considerable. The differences include the size, the proportions, and the drooping of the panicles.

The drooping of the panicle is caused primarily by the bend of the main axis and to a lesser degree by the downward recurving of the panicle branches appearing only in the large panicles with long branches.

The main axes of panicles in *D. glomerata* are differently curved. So far as the bend of the axis is concerned five types of panicles have been distinguished. They are shown schematically in fig. 3. The first diagram shows the upright type of the panicle, the second an upright panicle with

only the end drooping, the third a semi-drooping panicle, the fourth a drooping panicle, and the fifth a strongly drooping panicle. Besides the five main types shown in fig. 3 there are all kinds of intermediate forms. The drooping of the panicles in the particular tufts is not uniform and in each tuft there can be differently drooping panicles according to two or even three neighbouring types illustrated in fig. 3. Therefore, in the case of this trait only its range of variation can be considered.

The panicles in *D. slovenica* are always drooping according to the types 3 to 5 in fig. 3, though at the time of blooming they are somewhat more erect.

The panicles in *D. glomerata* are erect varying within the range of types 1 to 3. They usually are of type 1, especially before and at the time of blooming. After blooming the most common panicles are of types 1 and 2 or even 3. Panicles of type 1 after blooming are very rare. Table 4 shows the distribution of the *D. slovenica* and *D. glomerata* individuals according to the degree of drooping after anthesis.

From what has been said above it is apparent that both forms have drooping panicles, but in *D. slovenica* the trait is much more strongly marked. Although, the ranges of variation of this trait in *D. slovenica* and

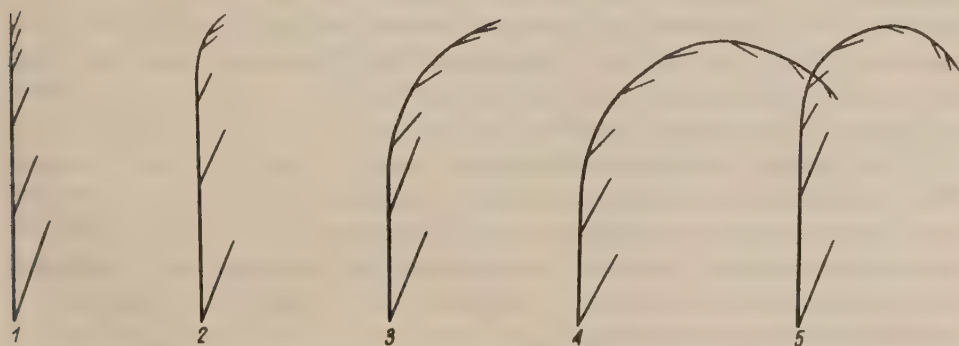


Fig. 3. Schematic diagrams showing the panicles in *D. slovenica* and *D. glomerata*: 1 and 2 — erect culms; 3 — semidrooping culm; 4 — drooping culm; 5 — strongly drooping culm

D. glomerata overlap, the drooping of the panicles can be accepted as one of the main traits distinguishing the two forms. The differences in this trait is the most distinct before anthesis, since then the panicles in *D. glomerata* are always erect and the panicles in *D. slovenica* are always drooping.

The size of panicles. The length of panicles in *D. slovenica* ranged 14.8 to 36.4 cm., the average values for the particular individuals being 18.0 to 28.7 cm.

Table 4

The drooping of the panicles according to fig. 3

No.	The types of the drooping panicles				
	1	2	3	4	5
<i>D. slovenica</i>			43 44 59	1 45 55 56 57 58 60	48
<i>D. glomerata</i> from lowland stands	2 3 41 42	18 61 62			
from mountain stands	33 34 37 49 51	32 35 38 39 40	31 35 63 63 64 65		

In *D. glomerata* the length of panicles ranged 9.3 to 23.0 cm. and the average length for the particular individuals was 11.6 to 19.3 cm. (Table 5).

The number of panicle branches was 5 to 7 in *D. slovenica* and 4.8 to 6.3 in *D. glomerata*. This small difference in the number of panicle branches coupled with the considerable differences in the length of panicles gives rise to an appreciable difference in the length of the internodes in panicles. The length of the internodes ranged 3.3 to 4.11 cm. in *D. slovenica* and 2.3 to 3.3 cm. in *D. glomerata*. The longer internodes and thus the greater distances between the panicle branches in *D. slovenica* make its panicles less compact and more outspread.

The longer the panicles the longer usually their lowest branches. The lowest panicle branches ranged 7.7 to 16.2 cm. in *D. slovenica* and 5.7 to 7.9 cm. in *D. glomerata*. Very interesting is the stability of the proportion between the length of the panicle branches and their part occupied by spikelets. The part occupied by the spikelets usually is 1/3 of the length of the branches, e.g.:

No. of plant	Length of the lowest panicle branch cm	Length of part with spikelets cm
65	12.4	3.8
1	7.7	2.7
55	10.0	3.8
59	16.6	5.2
18	5.7	2.2

The panicles in *D. slovenica* are distinctly longer than in *D. glomerata* and owing to their longer internodes they are less compact. Often the panicles of *D. slovenica* have numerous secondary branchings and two

or even three branches growing from one internode. The branching of the panicles in *D. glomerata* is never so profuse.

The spikelets. The differences between *D. slovenica* and *D. glomerata* in the structure of spikelets are not important. The spikelets are arranged in small bunches usually of four spikelets and the small bunches join together forming larger ones.

The length of spikelets ranged 6 to 8 mm. in *D. slovenica* and 5 to 9 mm. in *D. glomerata*. The number of flowers in a spikelet was 1 to 6 in *D. slovenica* and 1 to 7 in *D. glomerata*. As is to be seen in Table 5 one plant can have spikelets with a different number of flowers. This variation differs in the particular plants. The greatest difference was found in plant no. 49 which had six kinds of spikelets with 1, 2, 3, 4, 5, and 6 flowers. The variation was smallest in plant no. 1 which had five flowers in all the spikelets. No significant difference was observed between *D. slovenica* and *D. glomerata* in the number of flowers in the spikelets. In both forms spikelets with three or four flowers predominated.

The ciliation of the spikelets is considered to be an important systematic trait of *Dactylis glomerata* (Müntzig 1937 p. 191 and 1943 p. 136). In the course of this investigation it was found that ciliation could be entirely absent or could occur in various degrees. Discrimination has been made between individuals which were glabrous slightly ciliated, ciliated, and very strongly ciliated (Table 5).

In *D. slovenica* individuals with glabrous or slightly ciliated spikelets are the most common. This is in agreement with Domin's (1943) report in which on the ground of the ciliation of spikelets he defined *D. slovenica* from the Belanske Tatry massif as var. *subglabra* with two forms: f. *atrichosantha* having glabrous spikelets and f. *trichosantha* having slightly ciliated ones.

In the spikelets the cilia grow on the main rib of the glumes and sometimes also on the main rib of the lower lemma. The presence of the ciliation depending on the position of the spikelet in the bunch. Only the glumes and the lemmas situated on the outside of the bunches and not covered by other spikelets are ciliated.

The position of the spikelets in a bunch also affects the structure of the glumes: those lying on the outside of the bunches are always well developed, long, sometimes awned at the tip, with 1 to 5 ribs and the midrib ciliated, all green, and with only the margins membranous and sometimes frayed. The glumes covered by other spikelets are always badly developed, small and thin, without chlorophyll, and all membranous. The midrib often is difficult to distinguish.

Tab

The panicles and the spikelets in *D. slo*

No. of plants	Length of panicles cm	A Average length of panicles cm	Length of lowest panicle branches cm	Average length of lowest panicle branches cm
<i>D. slovenica</i>				
1	18,4—25,7	20,1	6,2—11,5	7,7
48	16,0—20,8	18,1	6,0— 9,7	7,7
55	16,1—22,2	20,4	6,6—12,0	10,0
56	14,8—22,5	19,3	6,4—11,3	9,5
57	15,2—36,8	26,6	6,0—18,7	13,4
59	23,1—38,2	28,7	14,1—20,0	16,2
60	18,9—30,3	25,3	9,4—16,9	13,7
<i>D. glomerata</i> from lowland stands				
2	12,9—18,0	14,9	6,1— 8,2	7,2
18	9,3—14,0	11,6	4,2— 8,0	5,7
41	10,1—19,3	14,8	5,2— 9,5	7,9
42	10,6—16,0	13,5	4,5— 8,6	7,1
61	14,1—23,0	19,3	4,5—10,3	7,2
62	14,0—19,7	17,2	5,4— 9,3	6,9
from Bukowina				
35	17,0—23,5	19,3	8,5—11,7	10,1
from Morskie Oko				
63—65	19,1—30,3	22,6	10,3—14,7	12,4
Natural hybrids				
52	13,6—23,0	18,5	5,8—12,5	9,0
53	13,9—24,4	17,6	6,6—12,0	8,4
54	14,6—17,3	15,8	7,0— 9,2	8,5
Artificial hybrids				
107	17,5—23,7	21,0	8,8—12,8	11,2
106/2	7,2—16,6	12,9	3,7— 7,9	6,1
106/3	11,0—16,5	14,2	5,4— 9,0	7,0
106/4	11,4—16,5	14,1	5,2— 8,1	6,5

le 5

venica, *D. glomerata* and their hybrids

B Average number of panicle branches	A : B Average length of panicle internode cm	Length of spikelet mm	Number of flowers in spikelets	Ciliation of spikelets
6,0	3,3	7—8	5	glabrous
5,0	3,6	6—9	2 3 4 5 6	„
6,0	3,4	7—8	2 3 4 5	„
5,5	3,5	7—8	4 3	slightly ciliated
6,9	3,8	6—7	2 3	ciliated
7,0	4,1	7—8	1 2 3	glabrous
6,0	4,3	6—8	3 4	slightly ciliated
5,0	2,9	7	3 4 5	very ciliated
5,0	2,3	6—7	1 2 3	slightly cil- iated
5,1	2,9	6—7	3 4	glabrous
4,8	2,8	6—7	3 4	„
6,3	3,0	7—8	6 7	„
5,2	3,3	7—8	4 5	„
5,0	4,4	6—7	1 2 3 4	slightly cil- iated
6,0	3,4	8—9	3 4	glabrous
5,3	3,5	5—8	1 2 3 4	slightly cil- iated
5,3	3,3	5—7	1 3 4 5	„
4,9	3,2	5—8	3 4 5	„
6,0	3,5	6—7	3 4 5	„
5,4	2,3	6—7	3 4 5	„
5,0	2,8	7	4 5	glabrous
4,8	2,9	7	3 4 5	„

The different possibilities in the development of the glumes according to the position of spikelets in the bunch are as follows:

1. in spikelets covering the bunch from the top both glumes are well developed and of the same length,
2. in spikelets with one side in and one out of the bunch the glumes are asymmetrical; the outside glume is well developed and the inner one covered by the other spikelets is reduced,
3. the spikelets inside the bunch covered on all sides by other spikelets have both glumes reduced,

The spikelets inside the bunch are always glabrous even in strongly ciliated plants.

Contrary to the glumes the structure of the lemmas in *Dactylis* is always uniform.

The structure of the glumes and the lemmas in *D. slovenica* and *D. glomerata* is the same.

EXPERIMENTS WITH THE CULTIVATED PLANTS

The *Dactylis* plants were cultivated in the Botanical Garden in Warsaw from 1956 to 1960. The observations and measurements carried out through all those years have shown that the individuals of *D. slovenica* have retained their original growth habit and size during the five years of cultivation. This also applies to other traits, such as the harshness of the culms, the ciliation of the spikelets, the colour, the swelling at the culm base, the drooping of the panicles etc. The behaviour of the cultivated individuals of *D. glomerata*, whether brought from the lowlands or from the mountains, was the same.

The blooming season. The developmental cycle in *D. glomerata* is not uniform. There are races which flower early and races which flower late: this variability was already described by Turesson (1929), Oldemeyer and Hanson (1955), Lackamp (1955), Kalton and Laffel (1955), and others. The blooming season is an important trait for the cultivation of grasses. The blooming of the lowland plants of *D. glomerata* cultivated in the Botanical Garden in Warsaw began at the end of May. The differences in the time of blooming between the particular tufts did not exceed ten days.

The blooming of *D. slovenica* in the Tatras begins during the first half of July, the actual date depending on the local ecological conditions and the altitude of the stand. In the Botanical Garden in Warsaw the blooming season of *D. slovenica* was in the middle of June, i.e. a month earlier than in the Tatras but almost three weeks later than *D. glomerata* from the lowlands.

The specimens of *D. glomerata* brought from the Morskie Cko valley

in the Tatras and some specimens brought from the mountain stands in Bukowina Tatrzańska flowered in the middle of June, similarly as *D. slovenica*. This means that the late blooming of *D. slovenica* is a trait characteristic of the montane plants in general and results from their somewhat later development than of the lowland plants. The slower rate of development is retained by the *Dactylis* individuals even when they are cultivated for five years in the lowlands under different climatic conditions.

The *D. glomerata* plants no. 49 and 59 brought to the Botanical Garden in Warsaw from the Holubyho Dolina valley in the Tatras flowered simultaneously with the other individuals of *D. glomerata* from the lowlands. They must have been newcomers in the mountains and had still retained in their development the rhythm of the lowlands. This result confirms Professor Hádač's personal communication that *D. glomerata* is not one of the natural constituents of the flora in the Holubyho Dolina valley and that the isolated individuals found in this valley must have been brought there by men and sheep.

The following plants were used for the crosses: *D. slovenica* plants no. 45 and 55, and the *D. glomerata* plant no. 41 from the Puszcza Białowieska forest. All the three plants were typical representatives of their forms. The chromosome number of these plants was 28. For the crosses the flowers were castrated or additionally pollinated: the latter method was mostly used because castration in *Dactylis* plants is very difficult and ineffective. In additional pollination the panicles of both plants were placed under one isolator and in this way F_1 seeds and selfs were simultaneously obtained from one panicle. The morphological traits of the hybrids and the selfs were sufficiently different to distinguish between them.

The following crosses were carried out by the castration method:

No. 106 = 55 $\times$ 41 giving 4 seeds and 3 seedlings.

No. 107 = 41 $\times$ 55 giving 4 seeds and 1 seedling.

The plants from these crosses were unquestionable hybrids. Their chromosome number was 28.

The additional pollination method was used for three crosses the results being as follows:

No. of cross	Number of seedlings obtained from the cross		
	total	selfs	hybrids
108 = 41 $\times$ 55	24	9	15
105 = 55 $\times$ 41	22	9	13
109 = 45 $\times$ 41	16	3	13
Total	62	21	41

As is to be seen the number of hybrids obtained with this method is twice the number of selfs, the reason being the partial self-sterility of the parental plants. The partial self-sterility in *D. glomerata* was reported by Nilsson (1934), Myers and Hill (1943), Oldemeyer and Hanson (1955), and others. The results of the crosses in this investigation indicate that partial self-sterility occurs also in *D. slovenica*.

The morphology of the F_1 generation. Morphologically the F_1 generation was not uniform regardless of whether it was obtained by the method of castration or by the method of additional pollination. In the classification of the plants only the most characteristic traits of the parental plants were considered. These traits were: the swelling at the culm base, the date of blooming, the drooping of the panicles before and after blooming, and the appearance of the panicles outspread as in *D. slovenica* or compact as in *D. glomerata*.

The manner in which these traits occur in the parental plants and in the F_1 individuals is compared in Table 6. The table lists all the F_1 individuals obtained by the method of castration and some obtained by additional pollination, the individuals with different combinations of traits being selected in the latter case.

The data in the table show that the F_1 plants may have either a phenotype intermediate between the parental forms or some traits identical with one of the parental forms. The culm base may have no swelling or the swelling may be as thick as or smaller than in *D. slovenica*. In the hybrids the blooming season is retarded as in *D. slovenica* and semi-drooping panicles predominate. The semi-drooping panicles before anthesis may be erect or drooping after anthesis. The panicles may be outspread, compact, or intermediate. Various combinations of traits are also possible e.g. the plants with no swelling of the culm base may have drooping or semi-drooping and outspread panicles and on the contrary plants with a distinctly bulbed culm base may have erect and compact panicles as in *D. glomerata*.

This lack of uniformity in the F_1 generation points to the possibility of the independent inheritance of the particular traits and at the same time reflects the great phenotypic plasticity within the particular individuals.

The plants from the natural stands used for the crosses must have been heterozygotes with regard to the traits here considered.

The progeny obtained by selfing the two *D. slovenica* parent plants were uniform and identical with the parental plants. On the other hand the selfs of the *D. glomerata* parental plant differed from each other. It seems, therefore, that the main source of the lack of uniformity in the F_1 generation was the *D. glomerata* plant no. 41 used in the crosses.

Table 6

The comparison the parental plants with the artificial and natural hybrids

No of plants		Swelling of the culm base	Begin- ning of the blooming season 1960	Drooping of the panicles*		General habit of panicles
				before anthesis	after anthesis	
Parental plants						
<i>D. glomerata</i>	41	without bulb	25.V.	1	1	compact
<i>D. slovenica</i>	45	bulbed	10.VI.	4	4	outspread
<i>D. slovenica</i>	55	bulbed	10.VI.	4	4	outspread
Artificial hybrids F ₁						
	107	bulbed		3	3 4	outspread
	106/1	bulbed		1	1 2	compact
	106/2	intermediate		1 2	3 2	intermediate
	106/3	intermediate		4	4 3	outspread
	108/4	intermediate	8.VI.	3 4	3 4	outspread
	108/6	bulbed	8.VI.	3	1 2	outspread
	108/8	intermediate	8.VI.	1 2	3	outspread
	108/12	bulbed	12.VI.	1	1	compact
	108/13	without bulb	8.VI.	4	4	outspread
	108/15	without bulb	7.VI.	3	3	outspread
	108/16	without bulb	8.VI.	3	3	compact
Natural hybrids						
	46	intermediate	5.VI.	3	1 2 3	outspread
	47	bulbed	10.VI.	3	2 3	intermediate
	50	bulbed	5.VI.	4	1 2 3	outspread
	52	intermediate	5.VI.	1	1 2	outspread
	53	intermediate	7.VI.	2 3	2 3	compact
	54	without bulb	7.VI.	2 3	2 3 4	compact
<i>D. glomerata</i>						
from Morskie Oko						
	63—65	intermediate	10.VI.	3	2 3	outspread
from Bukowina						
	31	without bulb	13.VI.	3	1 2 3	compact
	35	bulbed	13.VI.	3	2 3	outspread
	39	intermediate	13.VI.	2 3	1 2 3	outspread
	40	without bulb	13.VI.	3	1 2 3	intermediate

* According to fig. 3.

Natural hybrids. The natural hybrids found in Holubyho Dolina in the Tatras closely resemble the F₁ hybrids obtained experimentally (Table 6). In the natural hybrids the swelling of the culm base may be small, intermediate, or well developed as in *D. slovenica*. Their blooming season is distinctly retarded with regard to *D. glomerata*, but earlier than of *D. slovenica*. The panicles are usually semi-drooping and similarly as

in the experimental F_1 plants the panicles may be semi-drooping before anthesis and erect or drooping after anthesis. The natural hybrids manifest a similar phenotypic plasticity within an individual as the F_1 hybrids (Table 6).

The *D. glomerata* plants from mountain stands. The plants of *D. glomerata* from the Morskie Oko valley and from Bukowina Tatrzańska manifest some similarity with the natural and the experimental hybrids. The plants from the Morskie Oko valley are morphologically uniform, but have some traits of *D. slovenica* expressed in a small degree. They all have slightly bulbed culm bases and panicles semi-drooping before anthesis and semi-drooping or erect after anthesis. The size of the panicles is similar as in *D. slovenica*, whereas the growth habit of the plants is that of *D. glomerata* (Table 6).

Some plants from Bukowina Tatrzańska also are somewhat like *D. slovenica*. The most similar is the plant no. 35: it has a distinctly bulbed culm base, semi-drooping panicles of almost the same size as in *D. slovenica*, and the vigorous growth habit characteristic for *D. slovenica*. In the other plants from this stand the swelling of the culm bases is intermediate, sometimes the panicles are semi-drooping and some plants have the vigorous growth habit (Table 6).

Although the plants in the Morskie Oko valley and in Bukowina Tatrzańska do not meet directly with specimens of *D. slovenica*, since its distribution in the Tatras is restricted to the Belanske Tatry massif, nevertheless, there are among them individuals which morphologically resemble the hybrids. The possibility that *D. slovenica* genes were transferred from the distributional areas of this forms in the Belanske Tatry massif to such neighbouring areas as the Morskie Oko valley and Bukowina Tatrzańska cannot be discarded as improbable.

CYTOLOGICAL EXAMINATIONS

D. slovenica is a tetraploid plant with the chromosome number $2n=28$. In the course of meiotic divisions there may be tetravalents in diakinesis and in metaphase I. The tetravalents may form regular rings, rings twisted into the figure 8, or disrupted circles or chains. A similar occurrence of tetravalents was reported by Müntzing (1937) for *D. glomerata*. Only in one instance the Y arrangement of the chromosomes in a tetravalent was found.

The number of tetravalents in one cell in *D. slovenica* ranges 0 to 6. Not one cell was found in which all the chromosomes would form tetravalents, but in all the plants an appreciable proportion of cells contained 14 regular bivalents. The proportion of cells in *D. slovenica* with 14 bi-

valents in diakinesis ranged 16.9 to 24.5 per cent, whereas the average number of tetravalents in one cell was 0.97 to 1.43. Besides the tetravalents and the bivalents the cells of *D. slovenica* may contain a few univalents and very rarely single trivalents (Table 7).

For comparison cytological examinations were also carried out on the *D. glomerata* plants no. 18, 41, 42, 61, and 62. Similar chromosome arrangements were found in diakinesis. The average number of tetravalents in one cell was 0.96 to 1.28 and the proportion of cells with bivalents only ranged 20.0 to 33.3 per cent. Only the plant no. 2 from Leba strongly diverged from these average values: the average number of tetravalents to one cell in this plant was 1.89 and only four cells with 14 bivalents were found in it. It is possible that these four cells were wrongly classified, because the distance between the chromosomes in late diakinesis is already considerable and the pairs are connected by one chiasma which often is almost invisible.

In the experimental F_1 hybrids (plants no. 105/18, 106/1, 106/2, and 106/3) and in the natural hybrid (plant no. 47) the average number of tetravalents in one cell ranged 1.01 to 1.28, whereas the proportion of cells with bivalents only was 15.0 to 33.3 per cent. These values were approximately the same as in the parental forms. Table 7 lists the numbers of tetravalents found in diakinesis of the pollen mother cells in *D. slovenica*, *D. glomerata*, and their hybrids. The table only lists the figure for euploid plants with 28 chromosomes.

The meiotic divisions in *D. slovenica* have an entirely regular course similarly as in *D. glomerata*. The meiotic divisions in all the pollen sacks of one flower are accurately synchronized.

However, deviations from the regular course of meiotic divisions have also been observed. In the 1st meiotic division there were occasionally 1 or 2 univalents in a cell, but more than two univalents were never found. In anaphase of the 1st division the univalents could either remain on the equatorial plate and not be included in the telophase nuclei or they moved to the poles and were then included in the telophase nuclei. When each of two univalents moved to a different pole then each telophase nucleus contained 14 chromosomes. The chromosomes were counted in the metaphase of the 2nd division. In one instance at this stage the arrangement of 13 and 15 chromosomes was found: this arrangement was probably the result of the inclusion of two univalents in one telophase nucleus of the 1st division.

In the 1st meiotic division dividing univalents were quite often found. Their division usually was retarded with regard to the bivalents separating in anaphase. In metaphase of the 2nd division 14 and 15 chromosomes were found in some instances. This distribution must have been the result

Tab.

The chromosome

No. of plants	2n	Number of counted PMC	Chromosome				
			14 _{II}	13 _{II} + 2 _I	12 _{II} + 1 _{III} + 1 _I	1 _{IV} + 12 _{II}	1 _{IV} + 11 _{II} + 2 _I
<i>D. slovenica</i>							
45	28	115	19	1	—	50	—
48	28	101	24	—	—	42	1
55	28	100	19	5	—	53	2
55 S ₁	28	199	49	—	—	62	3
<i>D. glomerata</i>							
2	28	101	4	—	—	35	—
18	28	141	48	—	—	62	2
41	28	101	18	2	1	44	2
42	28	101	26	—	—	37	2
61	28	100	22	—	—	46	—
62	28	100	27	—	—	51	—
Natural hybrid							
47	28	60	16	1	—	29	—
Artificial hy- brids F ₁							
105/18	28	110	37	2	—	38	—
106/2	28	102	22	3	1	50	1
106/3	28	100	21	2	—	34	—
106/4	28	100	15	—	—	49	1

of the division of one univalent in the first meiotic division. The simultaneous division of both univalents was also often observed.

In the 2nd meiotic division chromosomes left on the plate and not included in the telophase nuclei were frequent. The number of such chromosomes could be one or more and they were found in one as well as in both dividing cells.

In the tetrads additional micronuclei were sometimes found and they must have been formed from the chromosomes rejected in the 2nd division.

The same irregularities in the meiotic divisions as those caused by the presence of univalents in *D. slovenica* — i.e. chromosomes lagging on the plate in the 2nd division and the formation of micro-nuclei in the tetrads — were also found in *D. glomerata*. This means that the deviations from the regular course of meiosis in *D. slovenica* are of the same kind as the irregularities in *D. glomerata*.

The pollen fertility counts in the *Dactylis* individuals grown in the Botanical Garden in Warsaw were made in three consecutive years (1958, 1959, and 1960). The differences between the particular years were rather

1e 7

conjugation in diakinesis

conjugation in diakinesis							Average number of tetravalents per one PMC	PMC with bivalents only %
$1_{IV} + 1_{III} + 10_{II} + 1_I$	$2_{IV} + 10_{II}$	$3_{IV} + 8_{II}$	$3_{IV} + 7_{II} + 2_I$	$4_{IV} + 6_{II}$	$5_{IV} + 4_{II}$	$6_{IV} + 2_{II}$		
—	38	6	—	1	—	—	1,29	16,9
—	28	6	—	—	—	—	1,15	24,0
—	21	—	—	—	—	—	0,97	24,0
1	51	18	1	12	1	1	1,43	24,5
—	39	15	—	7	1	—	1,89	4,0
1	16	10	—	1	—	1	0,96	33,3
—	22	10	—	2	—	—	1,28	20,0
—	28	7	—	1	—	—	1,18	25,7
—	24	7	—	1	—	—	1,18	22,0
—	18	4	—	—	—	—	0,99	27,0
—	10	4	—	—	—	—	1,01	28,3
—	23	9	—	1	—	—	1,04	33,6
—	22	3	—	—	—	—	1,28	15,0
—	33	10	—	—	—	—	1,02	24,8
—	28	5	1	1	—	—	1,20	23,0

small, i.e. of the order of two per cent. Pollen fertility in the individuals of *D. slovenica* ranged 77 to 95 per cent and the pollen fertility range of *D. glomerata* plants from the lowlands and from the mountains was the same. The pollen fertility in the natural and the experimental hybrids was in no way reduced and was about 90 per cent.

DISCUSSION

In the polyploid complex of *Dactylis* the tetraploid forms are much more common than the diploid ones. The tetraploids are characterized by great expansivity and a much greater morphological variability than the diploids.

This is primarily caused by the lack of sterility barriers which allows the exchange of genes between the differentiated forms thus inhibiting the differentiation processes within the genus. The exchange of genes may take place even between the diploid and the tetraploid populations (Müntzing 1937, Zahory and Nur 1959).

The other reason for the great variability of the tetraploids is their tetraploid condition owing to which some genes can occur four times. This has been demonstrated by Brix and Quandt (1953) for the gene *g* determining the colour of leaves.

Many traits forming in *D. glomerata* continuous variation series — e.g. the growth habit of plants and the drooping of the panicles — must be determined by numerous genes. An interesting characteristic of the genus *Dactylis* is the presence in this genus of diploid and tetraploid individuals which are morphologically undistinguishable (Stebbins and Zahory 1959). This characteristic makes the classification of the genus very difficult. According to Domin's (1943) classification the whole genus *Dactylis* is one compound species with seven subspecies and numerous forms. Domin's classification is based on morphological traits and disregards the cytological differences although, already in 1930 Levan reported that *D. Aschersoniana* is a diploid plant as distinguished from the tetraploid *D. glomerata*. Domin, on the ground of morphological traits, assumed that the distribution of *D. Aschersoniana* extended over the whole of Poland. In the course of the present investigation plants morphologically identical with *D. Aschersoniana* were often found in central Poland, but they always had 28 chromosomes, whereas the diploid *D. Aschersoniana* probably occurs only in the beech forests of West Poland. In Domin's classification the plants with different chromosome numbers but morphologically identical are classified together as one subspecies.

Stebbins and Zahory (1959) also consider the genus *Dactylis* to be one compound species *D. glomerata*, but unlike Domin's classification their's is based on the chromosome number. Within the species *D. glomerata* they distinguish diploid and tetraploid subspecies. In this approach morphologically identical plants may be assigned to two different subspecies and the difficulties arising in this connection were pointed out by Stebbins and Zahory themselves (1959).

All that has been said above clearly shows how differently the different workers approach the same phenomenon and what deplorable effects this has on the systematics of the genus *Dactylis*. The question thus arises what criterion should be given priority in the classification of the genus *Dactylis*, the morphology of the plants or the chromosome number?

The discrimination between diploid and tetraploid plants greatly facilitates the classification, but it seems illogical to assign to different systematic units plants morphologically identical, when within the diploid or the tetraploid forms the taxonomic units are discriminated according to the morphological traits alone.

All this makes the studies on the genus *Dactylis* a very difficult, but at the same time a very interesting problem, all the more so as the group of plants covered by the genus is still in the course of the gradual differentiation of the particular forms.

An example of this differentiation is provided by *D. slovenica* related with *D. glomerata* by many common traits. The morphological traits of both these forms are compared in Table 8. As is to be seen from this table *D. glomerata* has a much wider range of variation than *D. slovenica*.

The data assembled in Table 8 can be divided into three groups:

In the first group are the traits manifesting a continuous variation; in *D. glomerata* the range of variation is wide and in *D. slovenica* much narrower and contained within the limits of *D. glomerata*. These traits are the growth habit of the tufts, the colour and the harshness of the plants, the ciliation of the spikelets, the length of the ligule, and the number of flowers in a spikelet.

The second group also consists of traits with a continuous variation, but the overlapping of the variation ranges in *D. glomerata* and in *D. slovenica* is only partial and may differ in various degrees. The overlapping is wide in the case of the leaf widths and the maximum culm lengths, and narrow in the case of the average panicle lengths; the ranges of variation of the average lengths of internodes and of the drooping of panicles only contact.

The third group includes the contrasting traits, i.e. the swelling of the culm base or its absence and early or late blooming season. In the case of these traits the variation is discontinuous.

The late blooming of *D. slovenica* cannot be regarded as a systematic trait, since it is characteristic for all montane plants, but it is useful in the classification of plants.

The most important for distinguishing between the particular forms are the contrasting traits and for this reason the swelling of the culm base is the most characteristic trait of *D. slovenica*.

The drooping of the panicles and the length of the internodes also are useful traits for distinguishing between the two forms, because their ranges of variation are different, but these traits do not occur only in *D. slovenica*.

D. Aschersoniana has drooping and outspread panicles similarly as *D. slovenica*, however, the differences between these two forms are very great. *D. Aschersoniana* is a small frail plant, whereas *D. slovenica* is remarkable for its strong and vigorous growth habit. Szafran (1933) in the report on his investigations on the grasses of the pastures in the East Carpathians gives a detailed description and the measurements of

Table 8

The morphological traits of *D. slovenica* and *D. glomerata* from lowland stands

A. The qualitative traits

	Types of the growth habit*	Colour of plants	Harshness of plants	Swelling of the culm base	Drooping of panicles**	Ciliation of spikelets	Blooming season in Botanical Garden in Warsaw
<i>D. slovenica</i>	3—4	pale green bright green	smooth slightly harsh	bulbed	3—5	glabrous slightly ciliated	middle of Juli
<i>D. glomerata</i>	1—4	pale green bright green bluish green grayish green	smooth slightly harsh harsh very harsh	without bulb	1—3	glabrous slightly ciliated very ciliated	end of May

B. The quantitative traits

	Maximum length of culms cm	Width leaves mm	Length ligule leaves mm	Average length of panicles cm	Average number of panicle branches	Average length of panicle internodes cm	Length of spikelets mm	Number of flowers in spikelets
<i>D. slovenica</i>	124—190	6—1	4—7	11,6—19,3	5,0—7,0	3,3—4,3	6—9	2—6
<i>D. glomerata</i>	96—136	4—12	3—8	18,1—28,7	4,8—6,3	2,3—3,3	5—8	1—7

* According to fig. 1.

** According to fig. 3.

what he believed to be *D. Aschersoniana*, but the data in his report indicate that the plants he described could not have been *D. Aschersoniana*. They probably were *D. slovenica* which by its outspread and drooping panicles resembled *D. Aschersoniana*.

The analysis of morphological traits seems to indicate that *D. slovenica* has developed from *D. glomerata*. The two forms are connected by series of continuous variations indicating that *D. slovenica* is a relatively young form still in the course of differentiation out of the *D. glomerata* population.

On the other hand *D. Aschersoniana* is believed to be one of the ancestors of *D. glomerata* (Müntzing 1937, Myers 1948, Stebbins and Zahory 1959) and therefore the genes determining the drooping of panicles could have been transferred from *D. Aschersoniana* through *D. glomerata* to *D. slovenica*.

Another alternative is that *D. slovenica* appeared in the Tatras as a result of a cross between *D. glomerata* and *D. Aschersoniana* and if this was the case the exchange of genes between these two species could have taken place through triploid hybrids. An exchange of genes of this kind was observed by Zahory and Nur (1959) in Palestine where they found numerous triploids on the stands where the tetraploid and diploid *Dactylis* populations contacted. The chromosome number of some individuals in the progeny of these triploids was 28.

Whichever of these possibilities is more accurate, *D. glomerata* is certainly the main ancestor of *D. slovenica* and the Tatra Mountains undoubtedly constituted a convenient ecological niche in which the new form could develop. The conditions prevailing in the mountains must have had a decisive influence on this process.

D. slovenica certainly arose as a Carpathian ecotype of *D. glomerata*, but its morphological uniformity and the rather well marked differentiation are sufficient to classify it as a distinct systematic unit. Domin (1943) was fully justified when he classified *D. slovenica* as a systematic unit of equal rank with *D. Aschersoniana* and *D. hispanica*.

The question whether *D. slovenica* is to be treated as an independent species or as a subspecies of *D. glomerata* depends on what systematic approach to the genus *Dactylis* is chosen. If Domin's and Stebbin's opinion that the genus only consists of the compound species *D. glomerata* is accepted, then *D. slovenica* must be treated as a subspecies. But if other species than *D. glomerata* are discriminated in the genus, such as e.g. *D. marina* Borill, then *D. slovenica* also fully deserves the rank of an independent species.

Cytological examinations have revealed no differences between *D. slovenica*, *D. glomerata*, and their hybrids. In all instances the course of

meiosis was similar and the average number of tetravalents in one cell was about one, which means that the level of tetravalents in *D. slovenica* is the same as in *D. glomerata*. These results confirm the earlier supposition that *D. slovenica* is a young form developed from the *D. glomerata* population and that it did not have time to become cytologically differentiated.

The values obtained in the course of this investigation for the level of tetravalents in *D. slovenica* and *D. glomerata* are very different from the data reported by other workers. The average number of tetravalents in one P.M.C. in *D. glomerata* was 3.5 according to M ü n t z i n g (1937), 2.8—4.4 according to M y e r s and H i l l (1942), and 3.5 according to M c C o l l u m (1958). The values reported by those workers are about three times the values obtained in this investigation. The difference probably arose owing to the high proportion of cells, more than 20 per cent, with bivalents only in the examined plants from the Tatras and the Polish lowland. This means that the differences in the level of tetravalents between the particular population of *D. glomerata* may be considerable.

M c C o l l u m (1958) found that the distribution of tetravalents in the tetraploid forms of the genus *Dactylis* follows a binomial curve. The result of this kind of distribution would be a constant level of about 3.5 tetravalents to one cell. M c C o l l u m found the same level of tetravalents in natural hybrids and in hybrids obtained experimentally by crossing two different forms. Nevertheless, in a few instances a lower average number of tetravalents in one cell was recorded.

It seems, therefore, that the average number of 3.5 tetravalents in one P.M.C. is the initial number for the tetraploid *Dactylis* plants. In some populations a reduction in the number of tetravalents and a corresponding rise in the number of bivalents takes place. This kind of chromosome behaviour is characteristic for segmental allopolyploids and makes possible further evolutionary changes in the genus *Dactylis*.

CONCLUSIONS

1. *Dactylis slovenica* is a tetraploid with the chromosome number $2n=28$ growing on limy substrata in the Carpathians.
2. *D. slovenica* crosses easily with *D. glomerata* giving fertile hybrids.
3. The course of meiosis in *D. slovenica*, *D. glomerata*, and their hybrids is the same. The average number of tetravalents in one P.M.C. in *D. slovenica* ranges 0.97 to 1.29. Similar numbers of tetravalents were found in *D. glomerata* and in the hybrids of the two forms.

4. *D. slovenica* must have developed out of *D. glomerata* the two forms being related by many common traits which differ only by their range of variation.

5. The main traits distinguishing *D. slovenica* from *D. glomerata* are the bulbed culm base and the drooping outspread panicles of the former form.

6. The plants of *D. slovenica* are morphologically rather uniform: they can be classified as a subspecies of *D. glomerata* on equal terms with the other already distinguished diploid and tetraploid subspecies.

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2. Urzędy pocztowe i listonosze
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