Bothalia 15, 3 & 4: 587–590 (1985)

The Kranz syndrome in the Eragrostideae (Chloridoideae, Poaceae) as indicated by carbon isotopic ratios*

HÉCTOR O. PANARELLO ** and EVANGELINA SÁNCHEZ†

Keywords: carbon isotope ratios, Eragrostideae, Kranz syndrome

ABSTRACT

$^{13}C/^{12}C$ ratios are generally regarded as being very reliable indicators of C_3 or C_4 photosynthesis. These relative carbon isotope ratios are expressed as a negative $\delta^{13}C$ and fall into two distinct groups: Kranz (or C_4) plants with δ between -9‰ and -18‰ and non-Kranz (C_3) plants with δ between -22‰ and -28‰. In this paper, 29 taxa, representing 12 genera, of the tribe Eragrostideae were examined by mass spectrometry for their $\delta^{13}C$ in dried leaf tissue. All these taxa proved to be C_4 plants with $\delta^{13}C$ values ranging between -13,6‰ and -10,9‰. These findings confirmed published leaf anatomical observations which showed that all the studied taxa had characteristic Kranz leaf anatomy.

INTRODUCTION

A syndrome of anatomical, cytological and physiological characters, all related to aspects of the carbon fixation process, has been reported from several families of Angiosperms. This syndrome has been called the Kranz syndrome, C_4 photosynthesis, and the Hatch-Slack pathway. Kranz plants exhibit a high degree of efficiency in the utilization of ambient CO_2, resulting in maximum photosynthetic production at high temperature and high light intensity. The anatomical characteristics of this syndrome have been known for 100 years (Haberlandt, 1882) but the physiological aspects have only been known for nearly 20 years (Kortschak *et al.*, 1965 and Hatch & Slack, 1966).

The anatomical specializations of the Kranz syndrome include the presence of a chlorenchymatous bundle sheath of large, thick-walled cells containing specialized chloroplasts distinct from those of the mesophyll — they are a greater size, a greater number per Kranz cell are present and they accumulate starch. In addition, the mesophyll cells adjoin the bundle sheath and are radially arranged. Plants possessing the Kranz syndrome fix carbon initially into four-carbon acids (oxalo-acetic acid, malic acid and aspartic acid) in these mesophyll cells (Hatch & Slack, 1970).

The only way to prove definitely that a plant is Kranz or non-Kranz is to investigate both its physiology, either directly, or indirectly by means of $^{13}C/^{12}C$ ratios, and to examine its anatomy, particularly the leaf anatomy. Differences in carbon isotope ratios between Kranz and non-Kranz plants are well documented (Fritz & Fontes, 1980). $\delta\ ^{13}C$ values for C_4 plants range between -18‰ and -9‰ with an average of about -12‰, whereas the $\delta\ ^{13}C$ for C_3 plants is between -38‰ and -22‰ with an average of -25‰ (Fig. 1). In the Poaceae, no ratios between -18‰ and -22‰ have been reported. Plants with Crassulacean Acid Metabolism also have high carbon isotope ratios (Fig. 1), but are not relevant in this study since CAM has not been demonstrated in the Poaceae (Brown, 1977).

The determination of carbon isotope ratios is a very convenient method for classifying plants as being Kranz or non-Kranz. Only small amounts of plant tissue are required, the data are very accurate and objective and the plant material need not be physiologically active. The use of dried specimens from herbarium sheets is, therefore, possible.

MATERIALS

Leaf tissue was taken from herbarium sheets. This plant material was obtained from many sources, and these are all gratefully acknowledged. Sources included: Dr Thomas R. Soderstrom, US National Herbarium, Smithsonian Institution, Washington, DC (US); Museo Argentino de Ciencias Naturales 'Bernardino Rivadavia' (BA); Herbario Gaspar Xuarez, Cátedra de Botánica, Facultad de Agronomía, Universidad de Buenos Aires (BAA); Instituto de Botánica Darwinion (SI); Instituto Miguel Lillo, Tucumán (LIL); Instituto de Botánica Agricola, INTA, Castelar (BAB); Instituto de Botánica, Facultad de Agronomía, Universidad Nacional del Noreste (CTES).

The genera of the Eragrostideae examined, arranged alphabetically, are given in Table 1. All voucher specimen sheets from which material was taken have been annotated with labels stating the $^{13}C/^{12}C$ ratio of that specimen.

METHODS

About 8 mg of the leaf samples were dried under vacuum at a temperature of 110°C for 8 hours or overnight. Every sample was then mixed with 100 mg vanadium pentoxide (V_2O_5) in a quartz vial and flame sealed under vacuum of about 10^{-4} mbar. The vial, containing the sample and the vanadium pentoxide (V_2O_5) was allowed to react at 1000°C in an

* INGEIS contribution No. 71

** Instituto de Geocronología y Geología Isotópica, Ciudad Universitaria, Buenos Aires, Argentina.

† Museo Argentino de Ciencias Naturales, 'Bernardino Rivadavia', Buenos Aires, Argentina.

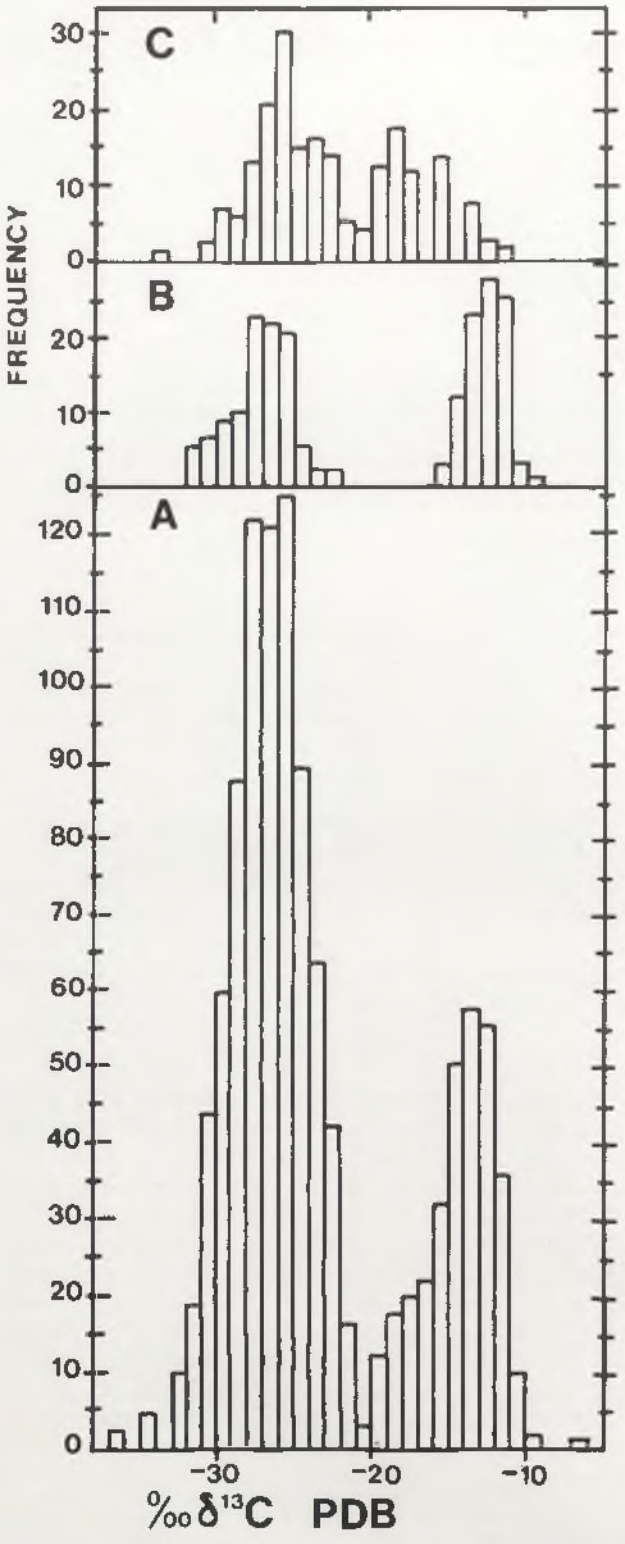


FIG. 1. — Carbon isotopic composition of photosynthetically fixed carbon; a, terrestrial plants; b, known C_3 and C_4 plants; c, known CAM plants. Reproduced from Fritz & Fontes, 1980.

electric furnace for five minutes, according to the technique described by Panarello *et al.* (1983).

The resultant CO_2 was introduced into the purification line by breaking the vial in a vacuum by means of a special 'vial breaker'. Water and light gases were removed and the CO_2 was transferred to a sample bottle by trapping with liquid nitrogen. The mass 45/44 ratio was then determined by measuring the samples in a Micromass 602-D Mass spectrometer. A reference and standard CO_2 from Carrara marble was used (Panarello *et al.*, 1980).

The results are expressed as $\delta\ ^{13}C$ (‰) defined as follows:

$$\delta\ ^{13}C = \left[\frac{Rs}{^{R}PDB} - 1 \right] \times 1000‰$$

Where Rs = Isotopic ratio of the sample
^{R}PDB = Isotopic ratio of the STANDARD PDB (Chicago PDB Standard, *Belemnitella americana* from the Cretaceous Pedee formation, South Carolina [Craig, (1954)].

The precision of measurement is ± 0,1‰ of the ratio. If $\delta\ ^{13}C$ is > 0 the sample is heavier than the standard, if less than 0 the sample is lighter, or depleted, in comparison with the standard. From this definition $\delta\ ^{13}C$ PDB = 0.

Carbon occurs in nature as two stable isotopes ^{12}C and ^{13}C, with the following average abundance:

^{12}C : 98,89%
^{13}C : 1,11%

However, the $^{13}C/^{12}C$ ratio is not constant and undergoes variations of a few parts per thousand in this ratio due to some physical and chemical processes. This variation in the isotopic composition is known as 'isotopic fractionation'. One of the most important processes in nature which causes isotopic fractionation of carbon is photosynthetic carbon assimilation by green plants.

Both terrestrial and marine plants have lower $^{13}C/^{12}C$ ratios than their respective carbon sources, atmospheric CO_2(δ~-7; -8‰) and ocean carbonates (δ ~ 0‰). This means that during photosynthetic CO_2 fixation there is preferential utilization of ^{12}C and exclusion of ^{13}C (Craig, 1957; Smith & Epstein, 1971).

RESULTS

The $\delta\ ^{13}C$ values of the taxa examined in this study are presented in Table 1. These carbon isotope ratios are also compared with published anatomical observations. As always, in the Poaceae, the $\delta\ ^{13}C$ values corroborate the leaf anatomy and all taxa have C_4 carbon isotope ratios (between -13,6‰ and -10,9‰) which agree with published accounts of Kranz leaf, and stem, anatomy for these taxa. This study has, therefore, confirmed the presence of the Kranz syndrome in these selected representatives of the Eragrostideae.

ACKNOWLEDGEMENTS

The authors are indebted to Dr Enrique Linares for facilities made available at the INGEIS and to the staff of the Stable Isotopes Laboratory. Miss Cristina Maetakeda (CONICET) is thanked for technical assistance and Dr R. P. Ellis (Botanical Research Institute, Pretoria) for reading the manuscript.

This research was supported with CONICET funds.

TABLE 1. — $\delta^{13}C$ values of selected representatives of the Eragrostideae

Species	$\delta^{13}C$‰	Kranz leaf anatomy	Voucher specimens
e benthamiana (S. Watson) Hackel	−10,9	Brown, 1958; Cáceres, 1950,51; Metcalfe, 1960; Sanchez, 1983a	Soriano 1056 (BAB)
bigelovii (S. Watson) Hackel	−13,2	Brown, 1977; Sanchez, 1983a	J. and Ch. Reeder 5833 (US)
hitchcockii Lahitte	−11,3	Sanchez, 1983a	Castellanos s.n. (BA 33421)
kingii (S. Watson) Hackel	−12,5	Sanchez, 1983a	J. and Ch. Reeder 5292 (US)
n aegyptium (L.) K. Richt.	−12,7	Brown, 1977; Metcalfe, 1960; Sanchez, 1974	Cabrera *et al.* 27474 (BAA)
lchella (H.B.K.) Willd. ex Rydberg	−12,3	Brown, 1977; Sanchez, 1979a, 1983b	J. Beatley 4886 (US)
glomerata (Walter) Burkart	−11,6	Sanchez, 1978	Hauman s.n. (BA 38750)
(L.) Gaertner	−11,8	Brown, 1958, 1977; Sanchez, 1974	W. Partridge s.n. (BA 60697)
chya (Lam.) Lam.	−11,6	Sanchez, 1974	W. Partridge s.n. (BA 59838)
enaceum var. *kurtzianum* (L.R. Parodi) Anton	−13,0	Sanchez, 1979a	Kurtz 9850 (BAA)
" var. *longiglume* (L.R. Parodi) Anton	−11,7	Sanchez, 1979a	A.T. Hunziker 2636 (BA)
" var. *pygmaeum* (Hackel) Anton	−11,8	Sanchez, 1979a	Castellanos s.n. (BA 19995)
osum var. *aristiglumis* (Caro) Sanchez	−11,7	Sanchez, 1979a	Covas 2071 (SI)
" var. *longearistatum* (Kurtz) Anton	−12,6	Sanchez, 1979a	Castellanos s.n. (BA 28/58)
" var. *mendocinum* (L.R. Parodi) Nicora	−11,6	Sanchez, 1979a	Perez-Moreau s.n. (BA 12497)
" var. *parodianum* Sanchez	−13,5	Sanchez, 1979a	Sánchez & Arriaga 1219 (BA)
" var. *pilosum* (Buckl.) Nash	−12,3	Brown, 1977; Sanchez, 1979a; Smith & Brown, 1973	G. Fisher 43041 (BA)
a (Griseb.) Vasey	−12,0	Sanchez, 1975	Castellanos s.n. (BA 24/1471)
ayensis (Kuntze) Parodi	−12,4	Sanchez, 1975	Castellanos s.n. (BA 10231)
Phil.	−11,3	Sanchez, 1984	De la Sota 224 (LIL)
na Griseb.	−12,6	Sanchez, 1984	J.H. Hunziker & O. Caso 4064 (BAB)
bens Phil.	−11,6	Sanchez, 1984	Hunziker & Krapovickas 5887 (BAB)
cina Phil.	−12,0	Cáceres, 1950; Metcalfe, 1960; Sanchez, 1984	Spegazzini s.n. (BAB 28237)
osa (Nutt.) Torrey	−12,5	Sanchez, 1984	B. Rohrer s.n. (LIL 286743)
otans (Michaux) Nicora	−12,7	Metcalfe, 1960; Nicora, 1962	H.B. Gephardt 1099 (BA)
evifolius Phil.	−13,6	Brown, 1977; Cáceres, 1950	C.G. Pringle (BA 9306)
Hitchcock	−12,2	Brown, 1977; Metcalfe, 1960; Smith & Brown, 1973	W. Benner 10295 (BA)
nsis Nees	−12,2	Sanchez, 1979a; Smith & Brown, 1973	H. Pueyo 87 (CTES)
tus (Nees) Ekman	−11,4	Brown, 1958; Brown, 1977	Molfino s.n. (BA 39580)

UITTREKSEL

Die $^{13}C/^{12}C$ verhoudings word algemeen as baie betroubare indikators van C_3 of C_4 fotosintetiese roetes beskou. Hierdie relatiewe koolstofisotoopverhoudings word as negatiewe $\delta^{13}C$ uitgedruk en val in twee duidelike groepe: Kranz (of C_4) plante met tussen -9‰ en -18‰ en nie-Kranz (of C_3) plante met δ tussen -22‰ en -28‰. In hierdie artikel is 29 taksons, wat 12 genera van die tribus Eragrostideae verteenwoordig, deur middel van massaspektrometrie vir hulle $\delta^{13}C$ in gedroogde blaarweefsel, bestudeer. Dit is bewys dat al hierdie taksons C_4 plante is met δ ^{13}C waardes wat varieer tussen -13,6‰ en -10,9‰. Hierdie bevindinge het gepubliseerde anatomiese waarnemings van blare, wat getoon het dat al die bestudeerde taksons kenmerkende Kranz blaaranatomie besit, bevestig.

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Bothalia 15, 3 & 4: 591–596 (1985)

The genus *Rubus* in South Africa. I. Chromosome numbers and geographical distribution of species

J. J. SPIES* and H. DU PLESSIS*

Keywords: chromosome numbers, geographical distribution, hybridization, polyploidy, *Rubus*

ABSTRACT

The geographical distribution of 14 of the *Rubus* species in South Africa is presented. Chromosome numbers of nine of the species were determined: six for the first time, one is confirmed and additional polyploid levels are described for the other two species.

It is demonstrated that the South African species of the subgenus *Idaeobatus* contain less diploid specimens and more polyploid specimens than their extra-African counterparts. This phenomenon could be attributed to hybridization between the subgenera *Eubatus* and *Idaeobatus*.

INTRODUCTION

The genus *Rubus* is spread over all continents and is found in most climatic regions. Focke (1910–1914) divided this genus into 12 subgenera of which only two are present in South Africa. All the representatives of the subgenus *Eubatus* Focke are introduced, whereas the subgenus *Idaeobatus* Focke contains both indigenous and introduced species.

Introduced *Eubatus* species all represent the subseries *Suberecti* of the series *Moriferi* and include the species *Rubus affinis* Wh. & N., *R. cuneifolius* Pursh., *R. pascuus* Bailey and *R. flagellaris* Willd. The introduced *Idaeobatus* species, *R. niveus* Thunb. and *R. phoenicolasius* Maxim., form part of the series *Nivei* of the section *Idaeanthi*. The indigenous *Idaeobatus* species include the sections *Rosifolii* (*R. rosifolius* Sm.), *Afromontani* (*R. immixtus* C.E. Gust.) and *Idaeanthi* [various species of the series *Afroidaei*, including *R. apetalus* Poir., *R. intercurrens* C.E. Gust., *R. longepedicellatus* (C.E. Gust.) C.H. Stirton, *R. pinnatus* Willd., *R. rigidus* Sm., *R. transvaliensis* C.E. Gust. and *R. ludwigii* Eckl. & Zeyh., with its subspecies *ludwigii* and *spatiosus* C.H. Stirton]. For the purpose of this study *R. adolfi-friederici* Engl., *R. ecklonii* Focke and *R. exsuccus* Steud. are included in *R. apetalus* Poir. because integration between these taxa renders the separation into distinct species impossible. Plants collected from a hybrid swarm in eastern Transvaal are included in *R.* × *proteus* C.H. Stirton.

The problems with *Rubus* taxonomy in South Africa are aggravated by the occurrence of apomixis, hybridization among indigenous species and between indigenous and introduced species, the variation produced by a breeding program and subsequent escape from cultivation of those plants and inadequate collected herbarium material. The aim of this study is, therefore, to provide cytogenetical evidence for the species delimitation in the South African species of *Rubus*. To achieve this goal, the results of a preliminary study on chromosome numbers and species distribution of the most important species are presented in this paper. Other papers in this series will include studies on meiotic chromosome behaviour, reproduction, hybridization and will be concluded with a cytotaxonomic study of the genus *Rubus* in South Africa.

* Botanical Research Institute, Department of Agriculture & Water Supply, Private Bag X101, Pretoria 0001.

MATERIALS AND METHODS

The plants used in this study were collected throughout South Africa and transplanted under quarantine in the Pretoria National Botanical Garden. The following 35 plants, representing nine different species, were used:

Eubatus

Rubus affinis:

TRANSVAAL. — 2329 (Pietersburg): Dap Naude Dam (–DD), *Stirton 5746*.

R. cuneifolius:

NATAL. — 2929 (Underberg): 14 km from Swartberg to Underberg (–CD), *Stirton 8154*. 2930 (Pietermaritzburg): 3 km from Midmar Dam to Lions River (–CB), *Henderson & Gaum 93;* 5 km from Pietermaritzburg to Mooi River (–CB), *Liengme s.n.* 3029 (Kokstad): 11 km from Harding to Weza (–DB), *Stirton 8102*.

R. pascuus:

TRANSVAAL. — 2430 (Pilgrim's Rest): 1 km from Graskop to Sabie (–DD), *Henderson & Gaum 18, Stirton 9800, 9861 & 9868*.

R. flagellaris:

TRANSVAAL. — 2530 (Lydenburg): Kaapse Hoop (–DB), *Henderson & Gaum 2*.

Idaeobatus

R. apetalus:

TRANSVAAL. — 2430 (Lydenburg): Kaapse Hoop (–DB), *Henderson & Gaum 6*.

NATAL. — 2929 (Underberg): Clairmont Plantation (–DD), *G. Hemm s.n. a & b*. 2930 (Pietermaritzburg): Endeni Farm (–CC), *Wells 5000*.

R. longepedicellatus:

TRANSVAAL. — 2430 (Pilgrim's Rest): 1 km from Graskop to Sabie (–DD), *Henderson & Gaum 22, Stirton 9862.* 2530 (Lydenburg): 5 km from Lydenburg to Sabie (–AB), *Henderson & Gaum 36;* Brooklands (–BA), *Henderson & Gaum 14.*

R. pinnatus:

TRANSVAAL. — 2530 (Lydenburg): Brooklands (–BA), *Henderson & Gaum 15.*

NATAL. — 3029 (Kokstad): 4 km from Kokstad to Weza (–CB), *Arnold 1335.*

R. ludwigii:

NATAL. — 2929 (Underberg): Kamsberg Nature Reserve (–BD), *Henderson & Gaum 41.*

CAPE. — 3226 (Fort Beaufort): Hogsback (–DB), *Admiraal & Drijfhout 2940.*

R. × proteus:

TRANSVAAL. — 2430 (Pilgrim's Rest): Bourkes Luck (–DB), *Henderson & Gaum 27, 28, 31 & 32;* Mac-Mac Falls (–DD), *Henderson & Gaum 20;* 1 km from Graskop to Sabie (–DD), *Stirton 9798, 9865 & 9866.*

NATAL. — 2929 (Underberg): 25 km from Himeville to Boesmansnek (–DC): *Henderson & Gaum 50 & 51.* 3029 (Kokstad): Ngeli Forest (–DA), *Stirton 8135.*

R. transvaliensis × R. longepedicellatus:

TRANSVAAL. — 2530 (Lydenburg): Nelspruit (–BD), *Henderson & Gaum 10.*

TABLE 1.—Chromosome numbers of some South African *Rubus* species

Species	Plant No.	2n=
Eubatus		
Rubus affinis	*Stirton* 5746	28
R. cuneifolius	*Stirton* 8102	14
	Stirton 8154	14
	Liengme s.n.	21
	Henderson & Gaum 93	28
R. pascuus	*Henderson & Gaum* 18	21
	Stirton 9800	21
	Stirton 9861	28
	Stirton 9868	28
R. flagellaris	*Henderson & Gaum* 2	28
Idaeobatus		
R. apetalus	*G. Hemm* s.n. a	14
	G. Hemm s.n. b	28
	Henderson & Gaum 6	28
	Wells 5000	28
R. longepedicellatus	*Henderson & Gaum* 22	14
	Henderson & Gaum 14	28
	Stirton 9862	28
	Henderson & Gaum 36	35
R. pinnatus	*Henderson & Gaum* 15	14
	Arnold 1335	28
R. ludwigii	*Admiraal & Drijfhout* 2940	14
	Henderson & Gaum 41	14
R. × proteus	*Henderson & Gaum* 28	14
	Stirton 9866	21
	Stirton 9798	28
	Henderson & Gaum 27	28
	Henderson & Gaum 32	28
	Henderson & Gaum 51	28
	Stirton 9865	35
	Henderson & Gaum 20	35
	Henderson & Gaum 31	42
	Stirton 8135	49
	Henderson & Gaum 50	56
R. transvaliensis × *R. longepedicellatus*	*Henderson & Gaum* 10	28
Rubus species	*Henderson & Gaum* 24	28

Rubus species:

TRANSVAAL. — 2430 (Pilgrim's Rest): 5 km from Graskop to Sabie (–DD), *Henderson & Gaum 24.*

Specimens are housed in the National Herbarium, Pretoria (PRE). Chromosome counts were made from meiotic squashes in aceto-carmine (Darlington & LaCour, 1976). Between 20 and 25 cells per plant were studied.

Distribution maps were obtained by using the data of all *Rubus* specimens available on PRECIS (Pretoria computerized information system) (Gibbs Russell & Gonsalves, in press).

RESULTS AND DISCUSSION

All chromosome numbers determined were multiples of 7 and somatic chromosome numbers of 14, 21, 28, 35, 42, 49 and 56 were observed in the investigated South African *Rubus* species (Table 1). Polyploidy occurred in six of the nine species studied. The different *Rubus* species varied in regard to their geographical distribution.

a) The subgenus *Eubatus*

The most widespread of the introduced *Moriferi* was *Rubus affinis*. Specimens representing *R. affinis* were collected in the northern Transvaal, Swaziland, Natal and southern and western Cape (Fig. 1). However, the western Cape is the only place where this species invaded the natural vegetation and where this species is regarded as a weed. The somatic chromosome number of 28 for *R. affinis* (Gustafsson, 1933, 1939 & 1943; Heslop-Harrison, 1953) is confirmed.

R. cuneifolius is restricted to Natal, with the majority of specimens collected in western Natal (Fig. 1). In addition to the chromosome number of 2n = 14 for *R. cuneifolius* reported by Shoemaker & Sturrock (1959), polyploid forms with 21 and 28 somatic chromosomes were observed during this study.

R. pascuus was collected in the eastern Transvaal (Fig. 1). This species frequently hybridized with *R. longepedicellatus* and the hybrids are included in *R × proteus*. Since *R. pascuus* contains both triploid and tetraploid specimens, it may either represent a hybrid rather than a parental form or a diploid form might be present in South Africa.

Two *R. flagellaris* specimens were collected in the eastern Transvaal (Fig. 1). This species has a somatic chromosome number of 28 in contrast to the published chromosome numbers of 56 (Faasen & Nadeau, 1976) and 63 (Einset, 1947).

The geographical distribution of *R. affinis* and *R. cuneifolius* (Fig. 1) indicates that these species occupy different habitats. This suggests that they were differently adapted to the South African climate. The single specimens of *R. affinis* collected in Transvaal, Swaziland and Natal might suggest separate introductions. The occurrence of polyploidy in the subgenus *Eubatus* is restricted to eastern Transvaal and Natal.

b) The subgenus *Idaeobatus*

The introduced species of the subgenus *Idaeobatus* have a limited distribution. *R. niveus* is restricted to Swaziland and the adjacent Transvaal areas (Fig.