Arthropod-Plant Interactions (2023) 17:855–862
https://doi.org/10.1007/s11829-023-09992-7

ORIGINAL PAPER

# Three-way interaction: severity of tufted grass fungal infection affects *Botanophila* fly reproduction

**Krzysztof Stawrakakis**[1] · **Zbigniew Kasprzykowski**[2] · **Marlena Lembicz**[1]

Received: 13 December 2022 / Accepted: 3 July 2023 / Published online: 1 August 2023
© The Author(s) 2023

## Abstract

The interaction between *Botanophila* flies and *Epichloë* fungi is one of the few interactions in which grass-associated fungi are 'pollinated' by flies. We analysed the gains and losses for both interaction partners and the time factors determining cost dynamics. A hypothesis regarding the relationship between changes in the severity of grass infection with *Epichloë* fungus, size of fungal sexual structures (stromata), and fly presence (expressed as egg and larvae numbers) was tested. During a 3-year study conducted on permanent field plots, fungal infection of wild grass tussocks of *Holcus lanatus* and fly visits to fungal stromata were monitored. An increase in the number of shoots with stromata and a reduction in stromata size were observed. From one year to another, flies deposited fewer eggs on stromata, and larval body mass decreased. We explain these results by the deterioration of grass condition due to increased *Epichloë* infection, which resulted in higher maintenance costs for the fungal parasite.

**Keywords** Anthomyiidae · Choke disease · *Epichloë* · Fungal endophyte · Stromata · Wild grass

## Introduction

The interaction between fungi of the genus *Epichloë* (Ascomycota, Clavicipitaceae) and flies of the genus *Botanophila* (previously *Phorbia*; Diptera, Anthomyiidae) was noted for the first time in the nineteenth century (Giraud 1872) and confirmed in the twentieth century (Lucas 1909, Trägardh 1913, Williams 1971). It was then observed that flies visit the fungal stromata to obtain food and lay eggs. The developing larvae feed on part of a stroma and thus are considered specialized parasites. After hatching from eggs in June, the flies undergo three larval instars before pupating in the soil close to the fungi host plants (Dušek 1970). Adults appear in the spring of the following year. *Epichloë* fungi are the only known food source for these larvae (Kohlmeyer and Kohlmeyer 1974). To date, six European *Botanophila* species and two additional species (Leuchtmann 2007) from North America associated with *Epichloë* fungi have been identified.

The ancestors of *Botanophila* flies associated with *Epichloë* fungi were probably herbivores feeding on grasses, similar to many modern representatives of the family Anthomyiidae (Bultman 1995; Bultman and White 1988). Individuals that feed on fungal reproductive structures (stromata) gain advantages because the stromata are a richer source of nutrients than the grass tissues (Bultman 1995). However, the *Epichloë–Botanophila* relationship is not limited to food issues alone. *Botanophila* females also lay eggs on *Epichloë* stromata and transfer spermatia of opposite mating types via their faeces. This procedure makes cross-fertilization of the obligate outcrossing fungus possible (Bultman and White 1988, Bultman 1995; Bultman and Leuchtmann 2008). The *Botanophila–Epichloë* relationship is based on the transport

This article is dedicated to the late Dr. Ziemowit Olszanowski (1961–2019), a zoologist (former employee of the Department of Taxonomy and Ecology of Animals, Adam Mickiewicz University in Poznań) and the first scientist in Poland who found the eggs and larvae of *Botanophila* flies in grassland at Pakość near Inowrocław.

Handling Editor: Dagmar Voigt.

✉ Marlena Lembicz
lembicz@amu.edu.pl

[1] Department of Systematic and Environmental Botany, Faculty of Biology, Adam Mickiewicz University in Poznań, Uniwersytetu Poznańskiego 6, 61-614 Poznań, Poland

[2] Department of Environmental Studies and Biological Education, Siedlce University of Natural Science and Humanities, 08-110 Siedlce, Poland

of resources and is similar to the relationship between pollinating insects and flowering plants (Bultman 1995). Thus, researchers recognized the *Botanophila–Epichloë* interaction as obligatory mutualism (Bultman et al. 1998).

The fungus incurs some losses because the larvae feed on the perithecia before releasing ascospores capable of infecting new grass individuals (Welch and Bultman 1993). The feeding behaviour results in a decrease in the number of discharged ascospores and, consequently, a reduction in fungal reproduction. However, even after larval feeding, the net production of reproductive tissues (perithecia) is higher for stromata receiving at least one *Botanophila* egg than those receiving none (Bultman et al. 2000).

More recently, we know that stromata fertilization may occur in *Botanophila* flies' absence (Rao et al. 2005, 2012; Hoffman and Rao 2014). Fertilization without the contribution of *Botanophila* vectors might occur through 'ascosporic fertilization', in which ascospores function as wind-transferred spermatia (Rao and Baumann 2004; Alderman and Rao 2008). Another possible explanation is that vectors other than *Botanophila* exist, such as slugs (Hoffman and Rao 2014; Rao et al. 2012; Bultman et al. 2022). It also cannot be excluded that a fly may not lay eggs or leave faeces whilst visiting a stroma. Yet, limited cross-fertilization may be possible through spermatia transferred externally on its body (Bultman 1995).

Each biological interaction has its own dynamics of gains and losses, influenced by many abiotic and biotic factors. Time is also a factor that significantly affects the strength of the interaction between organisms and grass and its effects (e.g. Lortie et al. 2004; Guisan and Thuiller 2005; Ferrier and Guisan 2006; Algar et al. 2009). The fly–fungus interaction depends on the third organism in the three-way interaction—the grass, an irreplaceable host for the fungi. In the stage of stroma development, the fungus is a grass parasite (Lembicz et al. 2010). With time, the host plant's condition deteriorates. According to the allocation principle (sensu Cody 1966), a plant inhabited by a fungal parasite must allocate more resources to defend against this parasite. A stronger antagonistic effect of fungi is also reflected in a more intensive assimilate uptake. Consequently, the costs of fungal maintenance increase, and a host plant has fewer resources available to develop fungi. As a result of this strain, its condition deteriorates. Resources are allocated to develop fungal stromata from leaves situated above these stromata. It may be expected that a weakened plant will produce smaller leaves. As a result, the fungus may absorb fewer resources than from shoots with larger leaves. This plant fitness loss may decrease the size of fungal reproductive structures—the stromata. In this study, conducted in a permanent field plot, the hypothesis was tested that an increase in infection severity caused by *Epichloë* fungus results in a decrease in its sexual structure (stromata) size in consecutive years and, consequently, the lower number of eggs laid by flies and the lower body mass of their larvae.

## Materials and methods

### Identification of fungus and flies

The endophytic fungus *Epichloë clarkii*, similar to other taxa of the genus *Epichloë*, belongs to the family *Clavicipitaceae.* This species was described for the first time on shoots of the grass *Holcus lanatus* L. (Poales, Poaceae) by White (1993). *H. lanatus* is a perennial grass that blooms from June to July. *E. clarkii* produces its reproductive structures (stromata) in the first weeks of June (Fig. 1). Taxonomic identification of the fungus and flies in the studied population of *H. lanatus* was conducted in our previous studies (Lembicz et al. 2013; Górzyńska et al. 2014). The identification of flies based on adults which had developed from eggs and larvae found on *E. clarkii* stromata. Two fly species were found and considered in the present study: *Botanophila phrenione* Séguy and B. *laterella* (Collin) (Pagel et al. 2019).

### Observations and measurements in a permanent field plot

The natural presence of the fungus *E. clarkii* and *Botanophila* flies in a wild population of the grass *H. lanatus* was monitored for 3 years. Monitoring was conducted on a permanent plot established in a meadow near the Faculty of Biology of Adam Mickiewicz University (AMU) in Morasko, Poznań (52°27.910′N, 16°55.370′E) (Fig. 2). Within an area of 25 $m^2$ divided into plots of 1 $m^2$, 19 tussocks of the grass *H. lanatus* infected with the fungus *E. clarkii* were selected and labelled in the first year of observation, and 20 tussocks were labelled in each of two consecutive years. The tussocks were selected from each corner of a plot. If there was no fungus-infected tussock in a given corner, then a tussock was selected from the plot centre. The weather conditions during the 3 years resembled each other.

In the first weeks of June, two groups of shoots were counted for each selected tussock: shoots without and with fungus and stromata, (predominantly) occurring on leaf sheaths. Next, one shoot with a stroma was cut from each of the labelled tussocks and put into a paper bag. After the transfer of samples to the laboratory of the Department of Plant Taxonomy at AMU, the following parameters were measured or counted: stroma length (i.e. the length of leaf sheath covered with fungal stroma), length of a leaf above the stroma, and number of fly eggs and larvae per stroma (if only a larva was found on a given stroma, it was accepted that an egg preceded it). All larval instars of both *Botanophila* species were pooled together. The presence of eggs

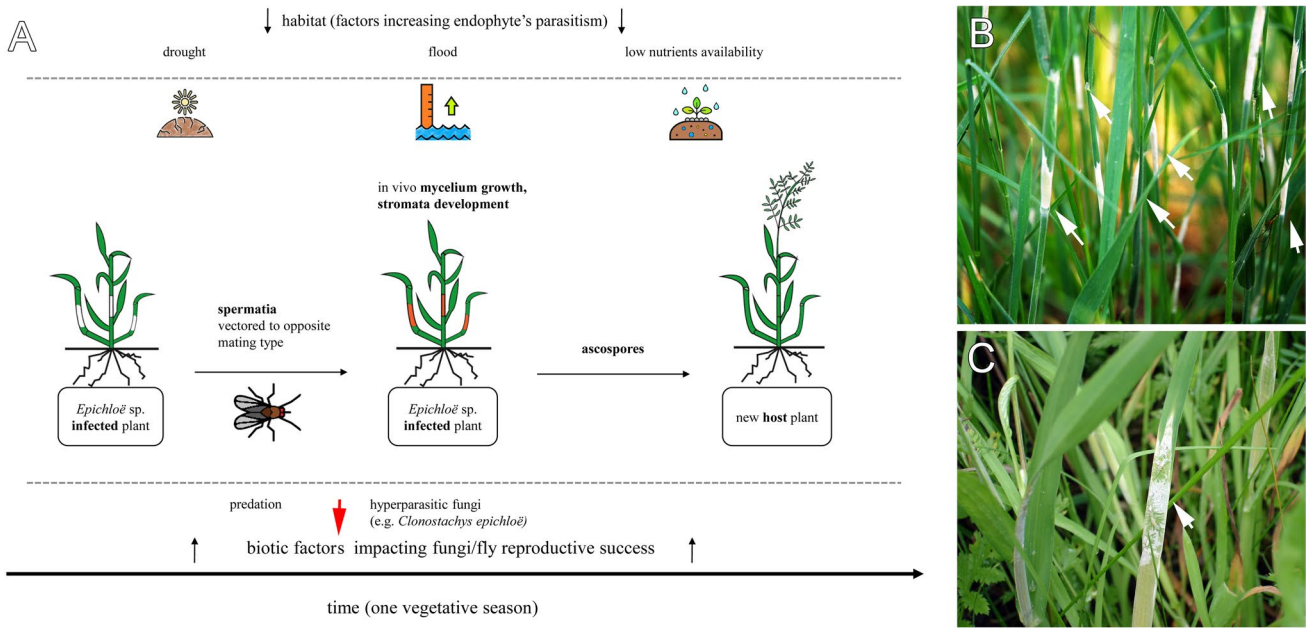



**Fig. 1** The diagram of the three-trophic grass–fungus–fly interaction and the abiotic and biotic factors, which were identified in the previous studies, affecting the gains and losses between the organisms that create these interactions (**A**). The grass acts as a host for the fungus *Epichloë* sp. (**B**), on which the fly lays its eggs and its larvae develop (**C**). The ascospores of the fungus, which can infect other grasses, form thanks to the flies, which, by carrying the reproductive spores, enable the start of sexual reproduction of the fungus. Arrows in **B** and **C** point to stromata of *Epichloë*

and larvae was checked under a stereo microscope (Olympus SZ61-TR; Olympus, Tokyo, Japan). Living larvae were collected from stromata and weighed with an analytical balance A&D, HM-120 (A&D Company, Ltd. Tokyo, Japan) with 0.0001 g accuracy. We assume to have sampled similar larval stages each year because of similar weather conditions and the same sampling dates.

### Statistical analyses

Factors that could affect the stroma length and larval body mass were examined using General Linear Models (GLMs) with an identity link function and a normal error distribution. The number of generative shoots, number of infected shoots, and leaf length in the first model and stroma length in the second model were used as continuous variables. To approximate the normal distribution, two predictors, the number of generative shoots and the number of infected shoots were logarithmically transformed. The study year in both models and the number of larvae in the second model were treated as categorical independent predictors. The number of larvae feeding on stromata was divided into three categories: 1—one larva on one stroma, 2—two larvae on one stroma, and 3—three larvae on one stroma. The relationship between the number of infected and non-infected shoots and between the stroma length and the leaf length was evaluated with a Pearson correlation. A one-way ANOVA and post hoc Tukey's test were used to detect differences in the number of eggs and the number of larvae between study years. The post hoc Tukey test also detected differences in larval body mass between the study years and between the categories of larval number. Only those results that showed a probability of $\alpha \leq 0.05$ were accepted to be statistically significant. All statistical analyses were carried out using Statistica ver. 12 (StatSoft 2018).

## Results

The GLM showed that the mean length of the stromata was affected by the leaf length and the study year ($R^2 = 0.86$, $F_{5,51} = 63.14$, $p < 0.001$, Table 1). The relationship between the stroma length and the leaf length was significant, strong, and positive (Pearson correlation, $r = 0.90$, $p < 0.001$, $N = 59$, Fig. 3). The third year of the experiment was characterized by a statistically lower value of stroma length than the first and second year (post hoc Tukey test, $p < 0.001$ for two cases, Fig. 4). However, no differences in this parameter were found between the first and second year (post hoc Tukey test, $p = 0.257$). The model also indicated that the number of infected and generative shoots did not affect the stroma length (Table 2).

Both the number of eggs and the number of larvae decreased during the experiment (Fig. 5), and significant

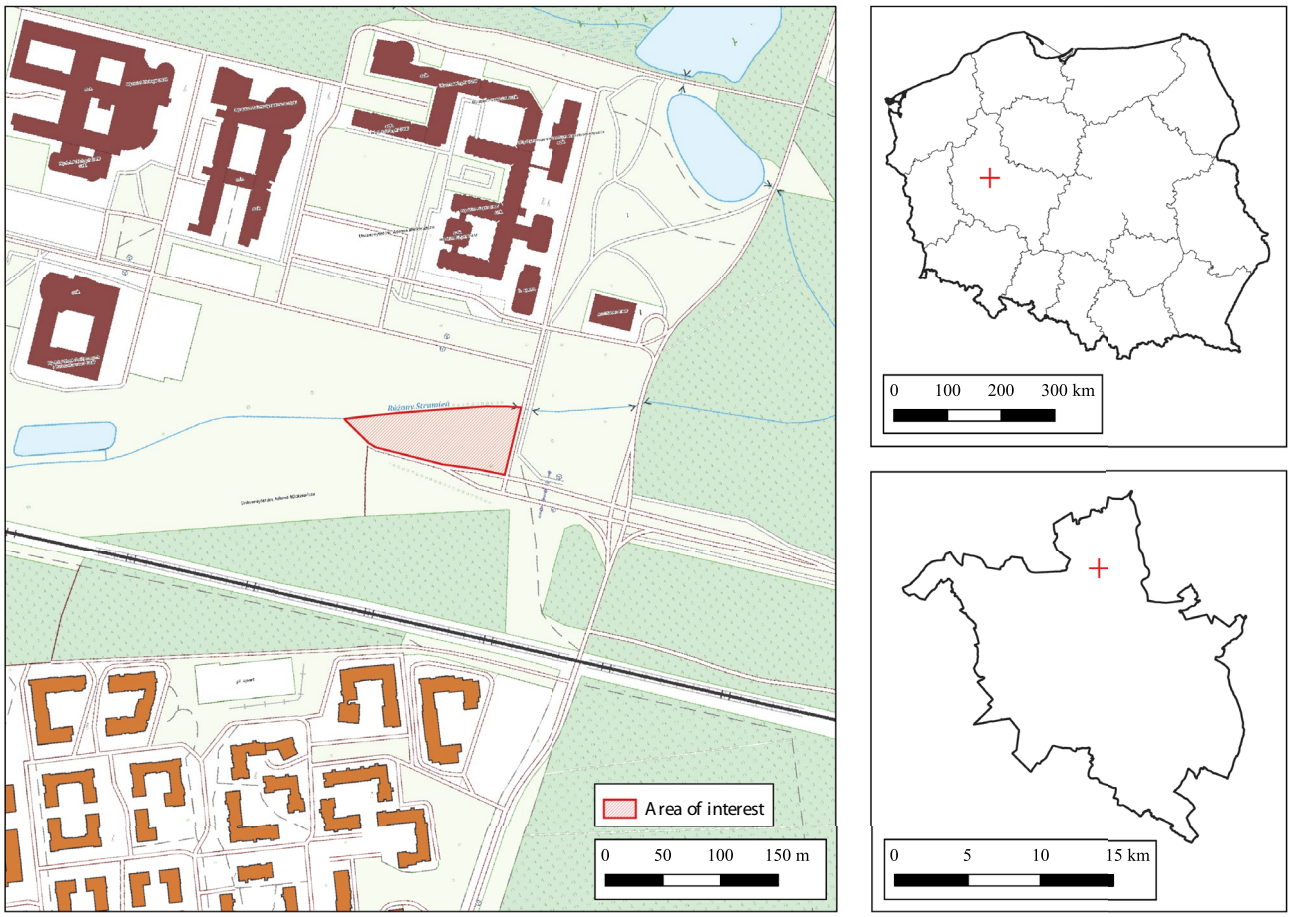



**Fig. 2** Map of the research area: **A**—part of the Morasko Campus UAM with highlighted research plot, **B**—map of Poland with the research location marked by a red cross, **C**—map of Poznań County with the research location marked by a red cross

**Table 1** Results of GLM analysing stroma length in the function of the year, number of generative shoots, number of infected shoots, and leaf length ($F_{5,51} = 63.14$, $p < 0.001$)

| Variables | df | *F* | *p* |
|---|---|---|---|
| Intercept | 1 | 7.59 | 0.008 |
| Year | 2 | 7.32 | 0.002 |
| Number of infected shoots | 1 | 0.86 | 0.359 |
| Number of non-infected Generative shoots | 1 | 0.79 | 0.377 |
| Leaf length | 1 | 75.67 | < 0.001 |

differences were found between study years (ANOVA for egg: $F_{2,56} = 45.53$, $p < 0.001$ and ANOVA for larvae: $F_{2,56} = 29.35$, $p < 0.001$). However, the post hoc Tukey test did not show differences in mean egg number between the first and second year ($p = 0.369$). In other analysed cases, both parameters differed significantly between study years ($p < 0.008$ for five comparisons).

The larval body mass was affected by the study year and the number of larvae but was not related to the stroma

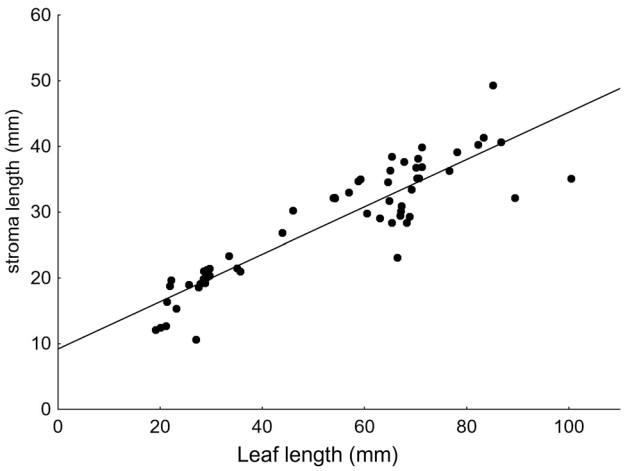



**Fig. 3** Relationship between the length of *Epichloë* stromata and the leaf length (Pearson correlation: $y = 9.20 + 0.36 \times x$, $r = 0.90$, $p < 0.001$, $N = 59$)

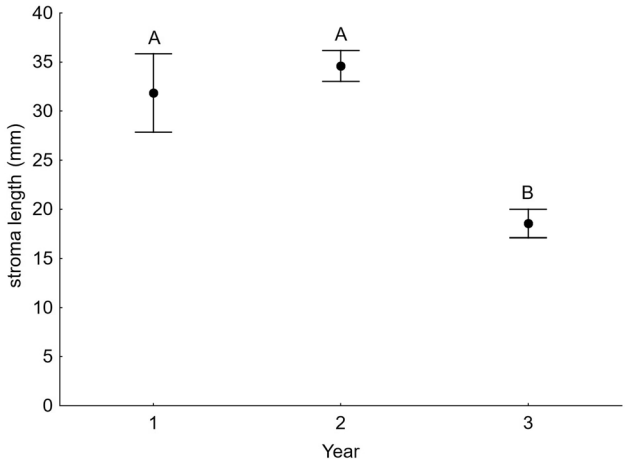



**Fig. 4** Length of *Epichloë* stromata during the 3 years of the study. The whiskers and dashed lines indicate the 95% confidence intervals**.** Means with different superscripts (A and B) differ significantly (post hoc Tukey test: $p < 0.001$)

**Table 2** Results of GLM analysing larval body mass in the function of the year, generative shoots, infected shoots, and stroma length ($F_{5,70} = 13.55$, $p < 0001$)

| Variables | df | *F* | *p* |
|---|---|---|---|
| Intercept | 1 | 13.49 | < 0.001 |
| Year | 2 | 16.38 | < 0.001 |
| Stroma length | 1 | 0.88 | 0.350 |
| Number of larvae | 2 | 19.02 | < 0.001 |

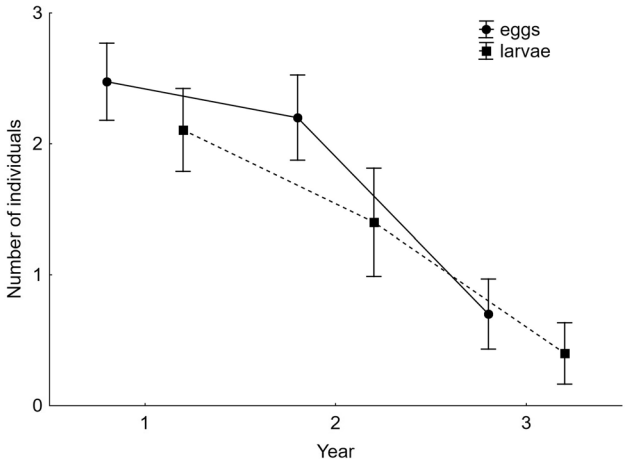



**Fig. 5** Number of eggs and the number of larvae of *Botanophila* found on stromata in the 3 years of the study. The whiskers and dashed lines indicate the 95% confidence intervals

length (GLM: $R^2 = 0.49$, $F_{5,70} = 13.55$, $p < 0.001$, Table 2). The larval body mass in the third year of the experiment significantly differed from the first year (post hoc Tukey

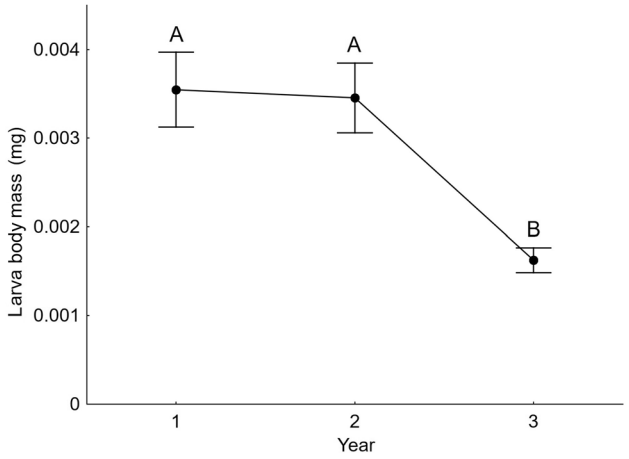



**Fig. 6** Body mass of *Botanophila* larva in the 3 years of the study. The whiskers and dashed lines indicate the 95% confidence intervals. Means with different superscripts (A and B) differ significantly (post hoc Tukey test: $p < 0.006$)

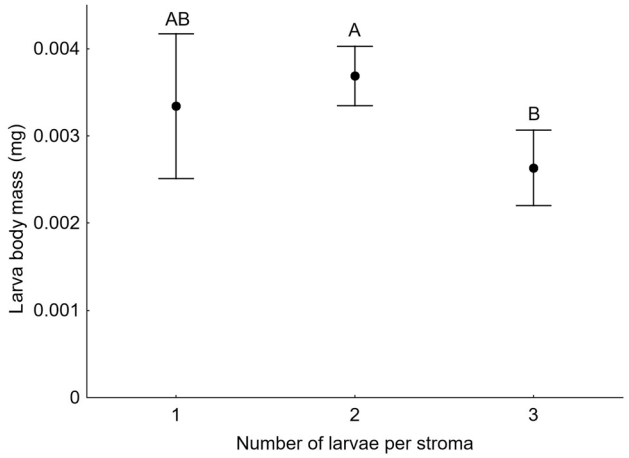



**Fig. 7** Body mass of *Botanophila* larva in three categories of larva number preying on stromata. Categories of the larva number: 1—one larvae on one stomata, 2—two larvae on one stromata and 3—three larvae on one stromata. The whiskers and dashed lines indicate the 95% confidence intervals. Means with different superscripts (A and B) differ significantly (post hoc Tukey test: $p = 0.017$)

test: $p = 0.004$) and the second year (post hoc Tukey test: $p = 0.006$, Fig. 6). The difference in larval body mass between the first and second year was not significant (post hoc Tukey test: $p = 0.950$). The mean larval body mass was lowest in the category of three larvae feeding on a stroma and differed only from the category of two larvae feeding on a stroma (post hoc Tukey test: $p = 0.017$, Fig. 7). The other two comparisons between the categories of larval numbers were not significant (post hoc Tukey test: $p > 0.176$).

## Discussion

In the 3-way interaction, the first step is an infection of the grass with *Epichloë* fungus. This fungus causes a well-known choke disease (Kohlmeyer and Kohlmeyer 1974), in which grass shoots are enclosed with fungal perithecia. The fungus in this form is a grass parasite that inhibits plant flowering and, consequently, seed production (e.g. Kirby and Santelmann 1964; Clay and Brown 1997; Lembicz and Olejniczak 2007; Li et al. 2020). The fungal infection in grass populations increased from year to year, which in the case of *Epichloë* infection has been previously described for different grass species populations (citation). In this study, we also found a time-related significant increase in the number of shoots infected with fungal stromata within the observed population of *Holcus lanatus*. This higher shoot number corresponds to an increase in food resources for flies.

For females and larvae of these flies, the fungus in the stroma form is the only food available (Bultman 1995). Thus, an increase in the number of eggs and larvae on fungal stromata might have been expected. However, vice versa, the present study showed a decrease in the egg number and larval body mass from year to year, i.e. with the growth of the *Epichloë* infection in the *H. lanatus.* The intensity of parasitic infection with endophytic fungi is manifested by the number of *Bothanophila* flies fed on the fungus-produced stromata. A higher number of stromata within a grass tussock worsened the condition of a plant, and as a result, it caused the production of smaller leaves in consecutive years. Each year, a plant grew older and became more strained by the increase in fungal stromata, metabolic process intensification, and oxygen-free radical content (Rozpądek et al. 2015).

We found a positive correlation between the leaf length situated above a stroma and stroma length. If the leaf was longer, the stroma was longer. With each year of the observation period, the leaf length decreased; thus, the resources essential for fungal maintenance decreased. As a result, fungal stromata decreased from year to year, and flies laid a smaller number of eggs on them. Furthermore, the larvae hatched from these eggs attained a lower body mass. Previous studies have shown that *Epichloë* stromata produce volatile substances that attract fly vectors (Schiestl et al. 2006; Steinebrunner et al. 2008) to increase the reproductive success of the fungus. The amount of produced volatile compounds depends on the stroma length. This relation may explain why more fly eggs were found on larger stromata (Górzyńska et al. 2011, 2014).

We are aware that a non-infected control is missed in our study and that climate conditions and other impacts might have influenced the observed tri-trophic community. Also, the grass could have suffered during the 3-year study period due to some variables not being studied, and the fungus could have tracked this degradation. However, a greater fungal infection could have led to host deterioration which could result in smaller stromata and reduced fly performance. Compared to the *Epichloë*-covered grass, the non-infected plants in the permanent field plots grew stronger without observable differences between the three study years.

In conclusion, the flies obviously responded to the appearance of shorter stromata in the grass population of *H. lanatus* by decreasing the number of eggs laid over a period of 3 years. This strategy (numerical response) of the flies might ensure sufficient food for the developing larvae. The opportunity for the flies to obtain food, which is essential for the development of the larvae, decreased with the decreasing stroma length. Furthermore, shorter stromata should produce fewer chemical compounds that attract the flies (Schiestl et al. 2006; Steinebrunner et al. 2008). As a result, the cost of the flies laying a continuously smaller number of eggs on the stromata may decrease the size of the fly population. We conjecture that in the three-way interaction, the grass condition became the biotic factor regulating the reproductive success of the fly. This interaction is particularly complex, taking place in the field in the evolutionary context. Likely, many factors combined during our research, and there was a significant decrease in the fitness of the fungus host—the grass. For the flies, this meant a reduction in the presence of food resources and, as a result, decreased fly reproductive success.

**Acknowledgements** We would like to thank Mr. Michał Delikta, MA (The Laboratory of Biological Spatial Information) for the preparation of the location maps of the research area. We are grateful to all the undisclosed reviewers and the Editor Dr. Dagmar Voigt for every comment, all of which made our manuscript better.

**Funding** This work was supported by the statutory funds of the Department of Systematic and Environmental Botany of Adam Mickiewicz University in Poznań, Project No. 0200000000/604/505000/BN002018/S/PB/0-25.

## Declarations

**Competing interests** All authors declare that they have no conflict of interest.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will

need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

## References


Alderman SC, Rao S (2008) Ascosporic fertilization of *Epichloë typhina* in *Dactylis glomerata* seed production fields in Oregon and implications for choke management. PHP. https://doi.org/10.1094/PHP-2008-0421-01-BR

Algar AC, Kharouba HM, Young ER, Kerr JT (2009) Predicting the future of species diversity: macroecological theory, climate change, and direct tests of alternative forecasting methods. Ecography 32:22–33. https://doi.org/10.1111/j.1600-0587.2009.05832.x

Bultman TL (1995) Mutualistic and parasitic interactions between *Phorbia* flies and *Epichloë*—convergence between a fungus and entomophilous angiosperms. Can J Bot 73:S1343–S1348. https://doi.org/10.1139/b95-396

Bultman TL, Lembicz M, Leuchtmann A (2022) Does the degree of mutualism between *Epichloë* fungi and *Botanophila* flies depend upon the reproductive mode of the fungi? J Fungi 8:1270. https://doi.org/10.3390/jof8121270

Bultman TL, Leuchtmann A (2008) Biology of the *Epichloë–Botanophila* interaction: an intriguing association between fungi and insects. Fungal Biol Rev 22:131–138. https://doi.org/10.1016/j.fbr.2009.04.003

Bultman TL, White JF Jr (1988) 'Pollination' of a fungus by a fly. Oecologia 75:317–319. https://doi.org/10.1007/BF00378616

Bultman TL, White JF Jr, Bowdish TI, Welch AM (1998) A new kind of mutualism between fungi and insects. Mycol Res 102:235–238. https://doi.org/10.1017/S0953756297004802

Bultman TL, Welch AM, Boning RA, Bowdish TI (2000) The cost of mutualism in a fly–fungus interaction. Oecologia 124:85–90

Clay K, Brown V (1997) Infection of *Holcus lanatus* and *H. mollis* by *Epichloë* in experimental grasslands. Oikos 79:363–370. https://doi.org/10.2307/3546020

Cody ML (1966) A general theory of clutch size. Evolution 20:174–184

Dušek J (1970) Präimaginale Stadien mitteleuropäischer Anthomyiiden (Diptera). 2. - Přirodovědné práce ústavů Ceskoslovenské Akademie Věd v Brně [2] 4(1):1–29, 7 + 4 pl.

Ferrier S, Guisan A (2006) Spatial modelling of biodiversity at the community level. J Appl Ecol 43:393–404. https://doi.org/10.1111/j.1365-2664.2006.01149.x

Giraud J (1872) Note sur les moeurs de l'*Anthomyia spreta* Meig. Ann Soc Entomol Fr 2:503–506

Górzyńska K, Lembicz M, Olszanowski Z, Leuchtmann A (2011) *Botanophila–Epichloë* interaction in a wild grass, *Puccinellia distans*, lacks dependence on the fly vector. Ann Entomol Soc Am 104:841–846

Górzyńska K, Olszanowski Z, Leuchtmann A, Lembicz M (2014) Oviposition preference of *Botanophila* flies (Diptera: Anthomyiidae) towards stroma size of *Epichloë* (Hypocreales: Clavicipitaceae) hosts. Ann Entomol Soc Am 107:532–538. https://doi.org/10.1603/AN13094

Guisan A, Thuiller W (2005) Predicting species distribution: offering more than simple habitat models. Ecol Lett 8:993–1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Hoffman GD, Rao S (2014) Fertilization of *Epichloë typhina* stromata by mycophagous slugs. Mycologia 106:1–7. https://doi.org/10.3852/13-069

Kirby B, Santelmann PW (1964) Germination and emergence of winged elm seed. Weeds 12:277–279. https://doi.org/10.2307/4040753

Kohlmeyer J, Kohlmeyer E (1974) Distribution of *Epichloë typhina* (Ascomycetes) and its parasitic fly. Mycologia 66:77–86

Lembicz M, Górzyńska K, Leuchtmann A (2010) Choke disease, caused by *Epichloë bromicola*, in the grass *Agropyron repens* in Poland. Plant Dis 94:1372

Lembicz M, Górzyńska K, Olszanowski Z, Michelsen V, Leuchtmann A (2013) The occurrence and preference of *Botanophila* flies (Diptera: Anthomyiidae) for particular species of *Epichloe* fungi infecting wild grasses. Eur J Entomol 110:129–134. https://doi.org/10.14411/eje.2013.018

Leuchtmann A (2007) *Botanophila* flies on *Epichloë* host species in Europe and North America: no evidence for co-evolution. Entomol Exp Appl 123:13–23. https://doi.org/10.1111/j.1570-7458.2006.00518.x

Li XZ, Simpson WR, ML, Bao GS, Niu XL, Zhang ZH, Xu HF, Liu X, Li YL, Li CJ (2020) Effects of seed moisture content and Epichloe endophyte on germination and physiology of Achnatherum inebrians. S. Afr. J. Bot. 134: 407–414. https://doi.org/10.1016/j.sajb.2020.03.022

Lortie CJ, Brooker RW, Choler P, Kikvidze Z, Michalet R, Pugnaire FI, Callaway RM (2004) Rethinking plant community theory. OIKOS. https://doi.org/10.1111/j.0030-1299.2004.13250.x

Lucas WJ (1909) Some points in the life history of Anthomyia spreta Mieg. Entomologist 42:241–242

Olejniczak P, Lembicz M (2007) Age-specific response of the grass Puccinellia distans to the presence of a fungal endophyte. Oecologia 152: 485–494. https://doi.org/10.1007/s00442-007-0660-z

Pagel L, Bultman TL, Górzyńska K, Lembicz M, Leuchtmann A, Sangliana A, Richards N (2019) *Botanophila* flies, vectors of *Epichloë* fungal spores, are infected by *Wolbachia*. Mycology 10:1–5. https://doi.org/10.1080/21501203.2018.1515119

Rao S, Baumann D (2004) The interaction of a *Botanophila* fly species with an exotic *Epichloë* fungus in a cultivated grass: fungivore or mutualist? Entomol Exp Appl 111:99–105

Rao S, Alderman SC, Takeyasu J, Matson B (2005) The *Botanophila–Epichloë* association in cultivated Festuca in Oregon: evidence of simple fungivory. Entomol Exp Appl 115:427–433. https://doi.org/10.1111/j.1570-7458.2005.00309.x

Rao S, Alderman SC, Kaser JM, Hoffman G (2012) Fertilization of *Epichloë typhina* in cultivated *Dactylis glomerata* by factors besides *Botanophila* flies. In: Young CA, Aiken GE, McCulley RL, Strickland JR, Schardl CL (eds) *Epichloë*, endophytes of cool season grasses: implications, utilization and biology. The Samuel Roberts Noble Foundation, Ardmore, pp 122–126

Rozpądek P, Wężowicz K, Nosek M, Ważny R, Tokarz K, Lembicz M, Miszalski Z, Turnau K (2015) The fungal endophyte *Epichloë typhina* improves photosynthesis efficiency of its host orchard grass (*Dactylis glomerata*). Planta 242:1025–1035. https://doi.org/10.1007/s00425-015-2337-x

Schiestl FP, Steinebrunner F, Schulz C, von Reuß S, Francke W, Weymuth C, Leuchtmann A (2006) Evolution of 'pollinator'-attracting signals in fungi. Biol Lett. https://doi.org/10.1098/rsbl.2006.0479

StatSoft Inc. 2018. Statistica, data analysis software system, version 12.0. https://www.statsoft.com

Steinebrunner F, Schiestl FP, Leuchtmann A (2008) Ecological role of volatiles produced by *Epichloë*: differences in antifungal toxicity. FEMS Microbiol Ecol 64:307–316. https://doi.org/10.1111/j.1574-6941.2008.00452.x

Trägardh I (1913) Bidrag till kännedomen om dipterlarverna. II. En svampätande Anthomyid-larv. *Egle* (*anthomyia*) *spreta* Meig. Ark Zool 8:1–16

Welch AM, Bultman TL (1993) Natural release of *Epichloe typhina* ascospores and its temporal relationship to fly parasitism. Mycologia 85:756–763. https://doi.org/10.1080/00275514.1993.12026329

White JF (1993) Endophyte-host associations in grasses. XIX. A systematic study of some sympatric species of *Epichloë* in England. Mycologia 85:444–455. https://doi.org/10.1080/00275514.1993.12026295

Williams CB (1971) Notes on a fungus-eating anthomyid fly in north Britain and North America. Entomologist 104:1–3

**Publisher's Note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.