

## Short communication

# “Pollination” of a fungus by a fly

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**Summary.** The mechanism resulting in fertilization of *Epichloë typhina*, a heterothallic ascomycete that is an endophytic pathogen of grasses, has now been discovered. Conidia of one mating type are produced in stromata and are then transferred by insects to individuals of the opposite mating type. One insect, *Phorbia phrenione*, is a particularly important vector of conidia. Once conidia of the opposite mating type have been transferred to a stroma, the life cycle continues with the formation of perithecia.

**Key words:** *Epichloë typhina* – Fungus-insect interactions – Pollination – Texas

While strobili of a few extant gymnosperms are visited by insects (Wester 1910), pollination by insects is generally restricted to the angiosperms (Baker and Hurd 1968). Insect-mediated fertilization outside the seed plants is virtually unknown. We report here the first documented case of insect “pollination” of an ascomycete fungus, *Epichloë typhina* (Fr.) Tul., by the fly, *Phorbia phrenione* Seguy.

*Epichloë typhina* is one of several species of ascomycetes in the order Clavicipitales that are endophytes of forage grasses (Siegel et al. 1985; Lyons et al. 1986). It infects grasses throughout the Poaceae (White et al. 1986, 1987a) and occurs in up to 80% of the individuals within a given host species (White 1987b). While the endophyte has been known for some time (Valimirkaya 1928 in Butler and Jones 1949; Sampson 1933), its prevalence in grasses is only now being discovered (White 1987a). The fungus is economically important due to production of ergot alkaloids (Lyons et al. 1986) that cause toxicoses in cattle foraging on grass containing the endophyte (Porter et al. 1981). Cattle feeding on tall fescue grass containing the endophyte experience weight loss or reduced weight gain (Lyons et al. 1981). In contrast, presence of the fungus in turfgrass may be economically advantageous due to reduced herbivory by insects (Funk et al. 1983).

The fungus spends most of its life cycle as somatic intercellular hyphae in ground tissue of leaves and stems of the host grass. Endophytic hyphae may also invade the embryo within the seed and thus infect individuals of the next host generation (Sampson 1933; White and Cole 1986).

As an endophyte of Canada wild-rye (*Elymus canadensis* L.), the fungus egresses to produce a white collar (stroma) on the sheath of grass stems. Conidia, thin-walled spores, are produced on the surface of the stroma. Within one week the conidial stroma may become covered with a layer of yellow-orange fruiting bodies (perithecia) in which ascospores are produced. Development of the ascospore phase of the life cycle is dependent upon transfer of conidia of one mating type to conidial hyphae of an unfertilized stroma of the opposite mating type; *E. typhina* is heterothallic (White and Bultman 1987). Conidia from stromata of the same mating type are incompatible; hence, transfer of conidia between different individuals (occurring on different individual host plants) is requisite for development of sexual maturity and production of ascospores. Uninfected grass hosts are presumably infected by ascospores, however this step remains little studied and poorly understood (Western and Cavet 1959).

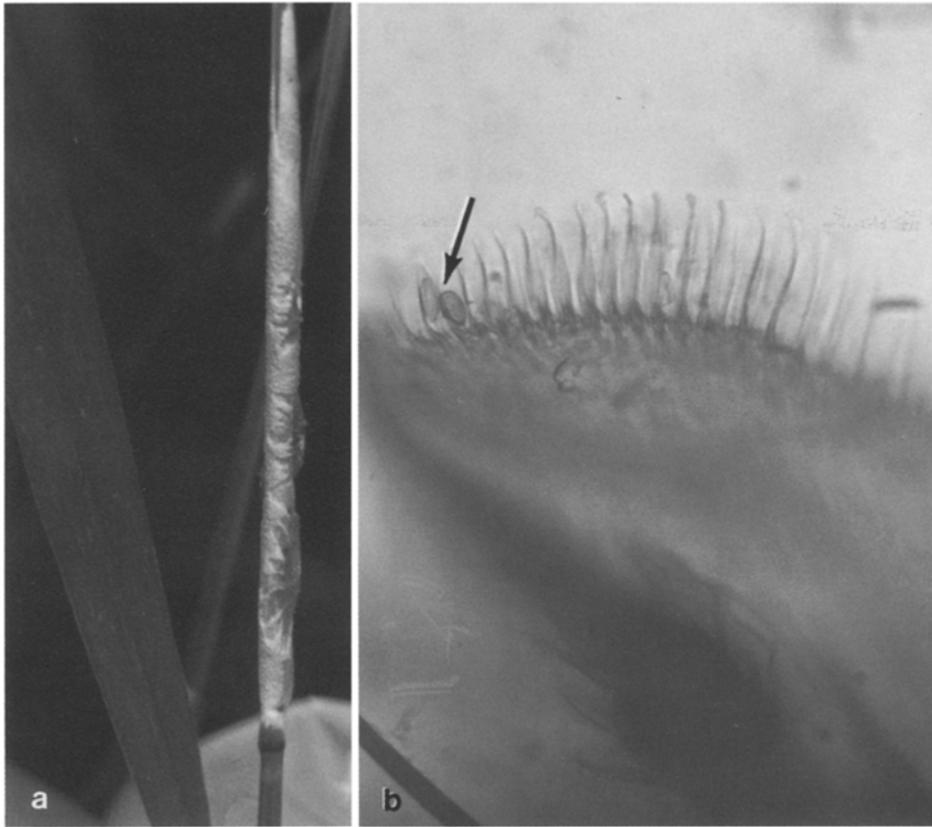
The monophagous fly, *P. phrenione*, obligately lays eggs on *E. typhina*. (The taxonomy of this and related species is currently under revision by G.C.D. Griffiths-University of Alberta.) Having found a stroma, flies ingest conidia while apparently testing the suitability of the stroma for oviposition (Kohlmeyer and Kohlmeyer 1974). One or more eggs are then laid and the fly leaves in search of another stroma. Larvae consume much of the stroma (Fig. 1a) before dropping to pupate in the soil and emerge as adults the following spring. Hence, the fly has been regarded as a hyperparasite (Kohlmeyer and Kohlmeyer 1974).

To better understand the relationship between the fungus and fly we monitored 38 individuals of *E. canadensis* at Brackenridge Field Laboratory (BFL) in Austin, Texas, USA, that possessed conidial stromata of *E. typhina*. We recorded which of these developed perithecia and which were occupied by eggs or larvae of the fly, *P. phrenione*. For comparison, we also monitored development of 118 conidial stromata in a population of *E. canadensis* near Caldwell, Texas. Here *P. phrenione* was apparently absent for no eggs or larvae were found on stromata nor were adults obtained through repeated efforts of sweeping vegetation.

Eighty-six percent of the conidial stromata at BFL developed perithecia (Table 1). Of these, 22 (66.7%) showed signs (eggs or larvae) of *P. phrenione* visitation. In contrast to the BFL site, only 19.5% of the conidial stromata developed perithecia at the Caldwell site, where *P. phrenione* was absent. *Phorbia phrenione* is thus implicated as an important

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**Fig. 1 a.** Stroma of *E. typhina* on stem of *E. canadensis*. Three brood chambers are present; larvae make feeding excursions from chambers creating feeding marks seen in stroma. **b** Conidia of *E. typhina* (arrow) adhering to tarsal spines of foreleg of a field-caught female fly (*P. phrenione*)

**Table 1.** Frequency of occurrence of perithecia on stromata at two sites\*

Site	<i>E. typhina</i> with perithecia	<i>E. typhina</i> without perithecia
Caldwell	23	95
BFL	33	5

\* Percentage of stromata that developed perithecia was significantly greater at BFL (where *P. phrenione* occurred) than at Caldwell (where *P. phrenione* was absent) 86.8% vs. 19.5%;  $P < 0.05$ ; test of two percentages; Sokal and Rohlf 1969)

vector of conidia of *E. typhina* but is apparently not the sole vector (Bultman and White, unpublished data).

To experimentally test the importance of transfer of conidia by insects we marked 12 individuals of *E. canadensis* with newly formed conidial stromata at BFL. Stromata were individually enclosed in mesh bags to prevent visitation by insects. Bags were removed after two months to check for sexual development (i.e., perithecia). None of 12 conidial stromata that were bagged with screening to exclude insects developed perithecia, yet 86.8% (33/38) of uncovered stromata formed perithecia (see above; Table 1). Wind velocity was likely lower inside than outside bags and this may have reduced possible transfer of conidia by wind. However, we feel wind is not a natural vector of conidia for the following reason. The Caldwell site (~1/2 h) contained  $\geq 1000$  stromata, of which only 19% developed perithecia (Table 1). If conidia were wind transported we would expect all or nearly all stromata to have been "pollinated". We feel the 19% were "pollinated" by insects other than *P. phrenione* (Bultman and White, unpublished work).

Our studies show when insects are excluded from stromata, ascospore producing perithecia are not produced, suggesting that insects transport conidia between stromata of *E. typhina*.

As a second and more conclusive test of the role of *P. phrenione* in the life cycle of the fungus we put five field-captured female flies individually into terraria that each contained six conidial stromata. Flies were allowed to visit stromata and lay eggs (average number of eggs laid per female was 7.6) for 24 h. Each fly was then individually transferred to another terrarium containing a single, newly-egressed conidial stroma (experimental). Flies were allowed to visit stromata for 2 h and were then removed. A similar stroma (control) was concurrently placed in a terrarium that lacked the fly. We then monitored experimental ( $n = 5$ ) and control ( $n = 5$ ) stromata for development of perithecia. All five experimental stromata developed perithecia, yet perithecia were lacking among all of the control stromata.

Our experiment clearly demonstrates that the fly in some way initiates the sexual phase in the life cycle of the fungus. We propose that the fly brings conidia of one mating type to a hypha of a conidial stroma of the opposite mating type, and thereby assists in fertilization of *E. typhina*, just as many insects carry pollen between flowers of different plants. That is, during oviposition flies effect union of a conidium with a compatible strain of mycelium that results in production of the ascus mother cell (which undergoes karyogamy and meiosis to form ascospores) much like pollen and ovum unite to produce seed in angiosperms.

To investigate the mechanism by which *P. phrenione* carry conidia, we inspected females under the compound microscope. We found conidia attached to legs (Fig. 1b) and mouthparts of field-captured females. No conidia were

found on the ovipositor. Observations that conidia pass viably through the fly's digestive tract (Kohlmeyer and Kohlmeyer 1974) suggest the fly may also "pollinate" stromate by defecation prior to oviposition.

This report is only the second documented case of obligate insect-mediated fertilization in the fungi. Spores of the heterothallic basidiomycete wheat rust, *Puccinia graminis*, are also transported by insects (Craigie 1927). Interestingly, aspects of the life cycle of *P. graminis* were understood in the nineteenth century (DeBary 1865), yet the importance of insects in its life cycle was not realized until much later. As with the rusts, understanding the life cycle of *E. typhina* has been slow. Its association with *P. phrenione* has been known for more than a century (Giraud 1872), yet until now the significance of the interaction went unnoticed. Elucidation of the life cycle of this economically important fungus may prove useful in efforts to manage its prevalence in populations of range grasses.

Due to the close association between *E. typhina* and *P. phrenione*, one might expect both to have coevolved with one another. Previous workers had considered the fly a parasite of the fungus (e.g., Kohlmeyer and Kohlmeyer 1974); it is now evident that the interaction is likely a mutualism. Apparent morphological features (T.L. Bultman, unpublished work) of both fly and fungus suggest the interaction is highly coevolved; not unlike some plant-pollinator systems. Outbreeding in *E. typhina* that is promoted by insect "pollination" may be the product of convergent evolution between the fungus and entomophilous angiosperms (Bultman and White, unpublished work). Of interest is whether the use of insect pollinators by both groups is the result of common selection pressures.

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