FERTILIZATION OF THE CHOKE PATHOGEN IN ORCHARDGRASS SEED PRODUCTION FIELDS IN THE WILLAMETTE VALLEY

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Introduction

Choke disease in orchardgrass seed production fields in the Willamette Valley is caused by an endophytic fungus, *Epichloë typhina*. The fungus, native to Europe, was inadvertently introduced into cultivated orchardgrass fields in western Oregon in the late 1990s (Alderman et al. 1997). In the presence of an abundance of host plants in close proximity, the fungus spread rapidly, and soon reached epidemic levels (Pfender and Alderman 1999; 2006). During vegetative growth of the orchardgrass, the fungus develops internally but when the plant switches to the reproductive phase, it proliferates externally forming stromata. These surround the inflorescence and, as a result, no seeds are produced on the affected tillers. Hence, the expression of the pathogen is called choke disease (Sampson and Western 1954).

Epichloë typhina has a self incompatible mating system in which transfer of spermatia between opposite mating types is required for fertilization and initiation of the sexual stage (White and Bultman 1987). Fertilized stromata are covered with a layer of orange fruiting bodies, the perithecia, in which ascospores are produced. In the wild, flies in the genus *Botanophila* are believed to transfer spermatia between fungal stromata of opposite mating types during oviposition (Bultman and White 1988). They drag their abdomen across the stroma, defecating spermatia in the process thereby facilitating fertilization of the fungus. An abundance of the fly species is present in association with *E. typhina* on orchardgrass in Oregon (Rao and Baumann 2004). However, surveys over three years indicated a lack of correlation between fly abundance and fungal fertilization (Rao and Baumann 2004). This suggests that, although flies are likely involved in fertilization of stromata, other mechanisms could also be responsible for fertilization of the choke pathogen in the Willamette Valley. Here, we document that ascospores, slugs and water splashes from rain can serve as alternative mechanisms for transfer of spermatia between opposite mating types of the choke pathogen in orchardgrass fields in Oregon.

Fertilization by ascospores

A laboratory experiment was conducted to determine if ascospores produced after fertilization of early emerging stromata can in turn fertilize subsequent stromata that emerge. Ascospores ejected from fertile stromata onto glass slides were suspended in water, and subsequently the suspension was transferred to unfertilized stromata (Alderman and Rao 2008). A similar suspension was prepared and transferred using conidia, and water was used as a control. A thickening of the stromata at the point of conidia or ascospore application was observed within 72 hours in all stromata inoculated with ascospores or conidia. There was no thickening or evidence of fertilization in stromata inoculated with only water. Ascospore germination from each stroma treated with conidia or ascospores exceeded 90% (Alderman and Rao 2008).

Based on these studies, in orchardgrass fields in western Oregon, ascospores from early-fertilized stromata could well facilitate subsequent fertilization of stromata. Earlier studies documented that fertilization commences in early May (Rao and Ackerman 2009). Hence, it is possible that *Botanophila* flies are responsible for fertilization of early emerging stromata, while early ascospores provide an alternative mechanism for subsequent fertilization. Also, a large number of ascospores are released from individual stroma in the region (Kaser et al. 2009); this could account for the rapid, widespread, and near complete fertilization of stromata observed in cultivated orchardgrass fields.

Fertilization by slugs

Slugs feed on choke stromata in orchardgrass fields in Oregon (Figure 1), and spermatia are present in their frass. To determine if the spermatia are viable and can fertilize the fungus, a frass transfer experiment was conducted with two slug species, the native *Prophysaon andersoni*, and the introduced *Deroceras reticulatum*. Frass from each species collected after exposure to an *E. typhina* stroma was transferred to a stroma of the opposite mating type, which was then examined after 10-14 days for signs of fertilization.

The fertilization response in the two slug species was significantly different. All frass transfers from *P. andersoni*, and 6 of 20 transfers from *D. reticulatum* resulted in fertilization (Figure 2). Thus, it is possible that spermatia that are consumed and excreted by slugs, especially the native *P. andersoni*, can serve as a vector for fertilization of the choke pathogen in cultivated orchardgrass fields in the Willamette Valley.

Fertilization through water splash

A greenhouse experiment was conducted to determine if water splash might facilitate fertilization of choke. Cages with two orchardgrass plants of opposite mating types (determined using molecular techniques) were randomly assigned to one of the following three treatments with six replications: water splash between plants, cotton swab between neighboring stromata, or no treatment (control). Observations made seven days later indicated significant differences in fertilization between the swab treatment and control, and the water splash treatment and control but not between the swab and water splash treatments. All swab replicates were cross fertilized, while 83.3% and

6.9% of splash and control replicates, respectively, were cross fertilized (Figure 3).

Thus, spermatia can be transferred between stromata of opposite mating types via water. The Willamette Valley receives frequent rains in winter and spring, and the close proximity of plants in cultivated orchardgrass fields could facilitate the transfer of spermatia via water splash between neighboring *E. typhina* stromata.

Conclusion

Based on these studies, the abundance of almost completely fertilized stromata even in the absence of fly eggs in the Willamette Valley could be due to diverse alternative mechanisms. Ascospores, which are present in abundance, are likely to contribute extensively to the near complete fertilization observed in the Willamette Valley. A second factor enabling fertilization is frass from slugs. Spermatia pass through slugs that feed on stromata in orchardgrass fields, and retain their viability and capability to fertilize the choke pathogen, indicating that slugs can fertilize stromata in a manner similar to that of flies. Finally, given the extensive rainfall in the region, and documentation that water splash can result in fertilization, rain is a third factor that can serve as an alternative mechanism for *E. typhina* fertilization.

References

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Figure 1. Slug feeding on a choke stroma on an orchardgrass tiller in the Willamette Valley.

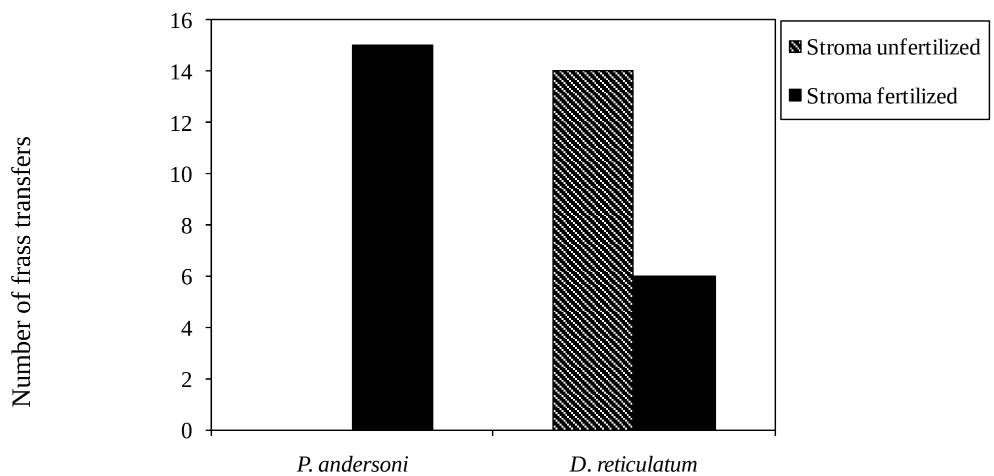

Figure 2. Fertilization of choke pathogen after transfer of frass from two slug species, *P. andersoni* (n=15) and *D. reticulatum* (n=20) that had fed on stroma of opposite mating type.

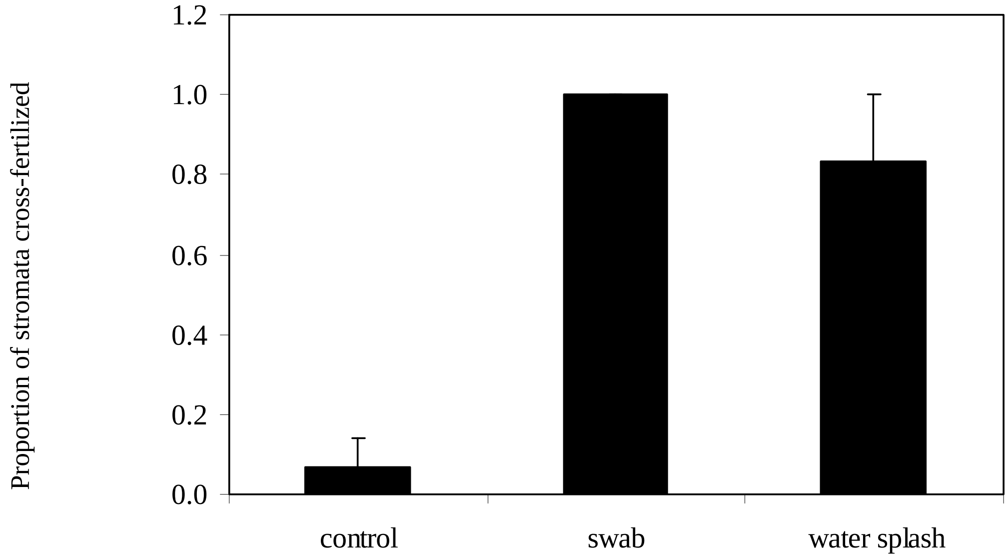

Figure 3. Proportion (+ SE) of stromata that developed perithecia in water splash experiment.