POTENTIAL CONTROL OF CHOKE IN ORCHARDGRASS WITH THE FUNGUS *DICYMA PULVINATA*

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Choke, caused by the endophytic fungus *Epichloe typhina*, is well established in the Willamette Valley. Plants are infected systemically and tend to remain infected throughout the life of the plant. Symptoms are expressed only during the reproductive phase of the plant, when the fungus proliferates on the immature panicle, restricting further panicle development. The fungal stromata that develop on infected panicles resemble small cattails (Figure 1), and these can be found in most orchardgrass fields in the Willamette Valley several weeks prior to harvest.

Figure 1. Stroma of *Epichloë typhina*.

During the summer of 2008, a fungus was found growing on stromata of *E. typhina* in a greenhouse at the USDA-ARS facility in Corvallis, OR. We identified the fungus as *Dicyma pulvinata*, a known pathogen of other fungi. It was subsequently found naturally occurring on stromata in an orchardgrass seed production field in the Willamette Valley. Infected stromata had fewer perithecia and appeared shrunken, desiccated, and pale gray to grayish-white, in contrast to the orange colored uninfected stromata with mature perithecia.

Greenhouse and field studies were set up to evaluate the potential of *D. pulvinata* as a biological control for choke in orchardgrass.

Greenhouse trials

To establish pathogenicity of *D. pulvinata* to *E. typhina*, ten healthy, unfertilized *E. typhina* stromata were sprayed with a conidial suspension (1 x 10^5 conidia/ml) of *D. pulvinata* (treated) and ten control stromata were sprayed with water (control) in a greenhouse. Conidia were collected in water from 3 week old cultures grown on PDA (potato dextrose agar). Just prior to inoculation, conidia were collected from stromata and redistributed among the stromata to fertilize them. Infected plants were grown from tillers removed from infected plants collected from an orchardgrass field near Corvallis, OR, two years earlier. Plants were maintained in the greenhouse and vernalized 12 weeks at 8°C with 8 h photoperiod in a growth chamber to induce reproductive tiller development, as required for initiation and development of stromata. On each plant, one to two stromata were sprayed with conidia or water. Inoculated and control plants were kept separate to avoid potential infection of controls from inoculated plants. All plants were misted by hand with a hand held sprayer twice a day with deionized water to encourage germination and growth of *D. pulvinata* on the stromata. Stromata were evaluated 4 weeks after inoculation. Assessments were made visually. The orange colored perithecia were clearly in contrast to the white unfertilized portions of the stromata. An assessment key with a series of drawings of stromata with various percentages of surface area covered (shaded) was made and used to determine the percentage stroma surface with mature perithecia. Following assessment, *D. pulvinata* was reisolated and the experiment repeated a second time. In each experimental run, a significant reduction in *E. typhina* perithecial development on surfaces of inoculated stromata was observed ($P < 0.5$, t-test) (Table 1) (Figure 2).

Table 1. Mean percentages (+/- standard deviation) of stromatal surfaces covered with perithecia 4 weeks after spraying unfertilized stomata of *Epichloë typhina* with *Dicyma pulvinata* (treated) or water (control).

Trial	Treated	Control
1	3.1 ± 8.8	82.5 ± 9.6
2	12.3 ± 16	58.8 ± 34.5

Figure 2. Stroma of *Epichloë typhina* colonized by *Dicyma pulvinata*.

Field plot trials

To evaluate the potential of *D. pulvinata* to prevent perithecial development in *E. typhina* under field conditions, a conidial suspension derived from 10 single spore isolates of *D. pulvinata* was prepared and sprayed on 12 unfertilized stromata in an established field plot of orchardgrass at the Oregon State University Hyslop Crop Science Farm near Corvallis, OR, on May 19, 2009. A small handheld sprayer was used to uniformly apply to runoff the conidial suspension or water control. The trial was repeated on June 4 using a second set of unfertilized stromata. In each repetition, an equal number of stromata were sprayed with water as a control treatment. All stromata were collected on June 24, stored under refrigeration and evaluated within 24 hours. Stromata were assessed as in the greenhouse trial described above.

A t-test was used to compare percentage stroma surface with perithecial development among treated and control treatments. A significant ($P < 0.05$) reduction in stroma surface fertilized was observed in stromata sprayed with *Dicyma* in trial 1 but not in trial 2. At collection, *Dicyma* was observed sporulating on 67% of inoculated stromata from trial 1 but none from trial 2. However, following incubation of stromata in moist chambers (petri dishes lined with wet tissue paper) for 72 h, *D. pulvinata* sporulated on 92 % of stromata from each of the two trials (Table 2).

Table 2. Percentage stroma surface fertilized and percentage stromata with *Dicyma pulvinata* in two experimental trials in 2009.

	Percentage stroma surface with perithecia		Percentage of stromata with *Dicyma pulvinata* sporulation	
Trial	Treated	Control	At collection	After 72 hr incubation
1	79 ± 9	90 ± 4	67	92
2	84 ± 9	83 ± 9	0	92

Discussion

Results from greenhouse and field trials indicate that *D. pulvinata* can cause a significant reduction in development of perithecia, although it is not yet clear to what extent perithecia would need to be reduced under field conditions to significantly impact the spread of choke within or between fields.

Dicyma pulvinata is widely distributed geographically, including North and South America, Europe, Asia, and Australia (Farr et al., 1989). In the U.S., *D. pulvinata* was recognized as a potential biocontrol agent of a leafspot disease of peanut, caused by *Cercosporidium personatum* (Mitchell et al., 1986, 1987). Studies of *D. pulvinata* in peanut demonstrated its sensitivity to a broad range of fungicides, but it is not known to what extent *D. pulvinata* would be impacted by fungicide sprays in orchardgrass.

There are currently no fungicide or cultural controls for choke in orchardgrass. The primary limitations to fungicide applications are in obtaining complete coverage of stromata, which are typically low in the canopy and covered with foliage, and in spraying stromata that emerge over an extended period of time. However, the ability of *D. pulvinata* to develop and spread among stromata could compensate for incomplete coverage if conidia are applied conventionally in a water suspension.

We suspect that development of *D. pulvinata* may be limited under dry conditions. This accounts for the difference in *D. pulvinata* development in mid May vs early June, in that much of June was unseasonably warm and dry. In additional studies this spring, we plan to determine whether *D. pulvinata* could reduce perithecial development if it is established in late April to early May, when stromata start emerging, and whether it could then spread to parasitize subsequent emerging stromata.

An intriguing aspect of *D. pulvinata* is how it would impact development of the *Botanophila* spp. fly larvae on the stromata. *Botanophila* flies are responsible for fertilization of stromata and their larvae depend on the fertilized stromata to complete their life cycle. Additional studies will need to be

conducted to better understand the interaction between *E. typhina*, *Botanophila* and *D. pulvinata* and the implications for choke management in orchardgrass.

References:

Farr, D.A., Bills, G.F., Chamuris, G.P., and Rossman, A.Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul. http://nt.ars-grin.gov/fungaldatabases/.

Mitchell, J.K., and Taber, R.A. 1986. Factors affecting the biological control of *Cercosporidium* leaf spot of peanuts by *Dicyma pulvinata*. Phytopathology 76: 990-994.

Mitchell, J.K., Jeger, M.J., and Taber, R.A. 1987. The effect of temperature on colonization of *Cercosporidium personatum* leafspot of peanuts by the hyperparasite *Dicyma pulvinata*. Agric. Ecosystems Environ. 18: 325-331.