PROSPECTS FOR ERGOT DISEASE MANAGEMENT WITH BIOCONTROL PRODUCTS

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Introduction

Ergot, caused by the fungus *Claviceps purpurea,* is a floral disease of grasses and a persistent problem in grass seed production systems in eastern Oregon and Washington. Ergot control is difficult due to the vast number of sclerotia that can remain in the soil after seed harvest (Dung et al., 2016). Use of biocontrol agents may reduce or delay sclerotia germination and reduce ergot infection by decreasing the abundance of sclerotia. Biocontrol research over the past 100 years has indicated that antagonistic microorganisms can act to inhibit disease occurrence and/or progression (McSpadden et al., 2002). The discovery of an effective biocontrol agent would provide another management option for integration into existing ergot management approaches in Pacific Northwest grass seed production systems.

Mycoparasitic fungi causing degradation of *C. purpurea* sclerotia have been previously reported (Ondřej et al., 2010). However, information is limited about the occurrence of microorganisms naturally associated with *C. purpurea* sclerotia in grass seed production regions of Oregon and Washington and their parasitic potential. This research focused on the discovery and evaluation of potential biocontrol options that can be readily incorporated into an integrated ergot disease management plan. Our research objectives were to: (1) isolate and screen naturally occurring microorganisms with potential to parasitize ergot sclerotia, and (2) evaluate commercial products for their ability to delay or reduce ergot sclerotia germination.

Materials and Methods

Isolation and screening of fungi and bacteria on *C. purpurea* sclerotia

A baiting technique was used to attract and isolate naturally occurring microorganisms that may be able to parasitize *C. purpurea* sclerotia. Fifty sclerotia collected from perennial ryegrass and Kentucky bluegrass were placed in nylon mesh bags and buried at a depth of 0.7 inch at ten different locations in a commercial perennial ryegrass field (PRG) and Kentucky bluegrass (KBG) field in Hermiston, OR and Madras, OR, respectively. After 1 month in the field, sclerotia were retrieved, washed, and cultured on either potato dextrose agar or nutrient agar media. In addition, for direct isolation, sclerotia obtained from commercial grass seed cleaning facilities were cultured in a similar manner.

Fungi and bacteria were isolated and identified using morphological and molecular tools. Isolates of *Epicoccum nigrum*, *Pantoea agglomerans*, *Pseudomonas putida*, and *Pseudomonas brassicacearum* were selected for screening based on their ability to inhibit sclerotia germination as documented in the scientific literature. Spore suspensions (10^9 CFU/ml) were produced by filtering pure liquid cultures of the isolates mentioned above through four layers of sterile cheesecloth or by washing spores from actively growing petri plates using sterile water.

The screening assay consisted of petri plates containing 20 g of autoclaved soil and 20 surface-sterilized sclerotia that were preconditioned in moist sterile soil at 41°F for 6 weeks to simulate vernalization and break dormancy. All treatments, consisting of *Epicoccum nigrum*, *Pantoea agglomerans*, *Pseudomonas putida*, *Pseudomonas brassicacearum,* and a water control, were replicated four times. Treatments were made using a hand-held sprayer, and plates were incubated at 60°F for 7 weeks after treatment. The number of parasitized and germinated sclerotia observed in each treatment was compared to the parasitized and germinated sclerotia of the water-treated control. Data were analyzed using ANOVA and means separated using Tukey's test.

Evaluation of commercial biocontrol products against sclerotia germination

A laboratory assay was conducted to evaluate the efficacy of commercially available biocontrol products, including Contans (*Coniothyrium minitans*; Advan LLC, Roswell, GA), Trichopel (*Trichoderma harzianum*; Agrimm Tech Ltd, Christchurch, NZ), SoilGard (*Gliocladium virens*; Certis USA LLC, Columbia, MD), and Serenade (*Bacillus subtilis*; Bayer CropScience, Research Triangle Park, NC) to inhibit sclerotia germination (Table 1).

This test consisted of 25 perennial ryegrass sclerotia placed on 25 g of sterilized soil contained in a petri dish.

Table 1. Trade name, active ingredient, concentration, formulation type, and application rates of commercial biocontrol products used in laboratory assays, 2016.

Trade name	Active ingredient and concentration	Formulation type	Rate
			(product/unit area)
Contans	*Coniothyrium minitans* (1×10^9 CFU/g)	Wettable granules	1.8 lb/a
Trichopel	*Trichoderma harzianum* (1×10^6 CFU/g)	Granular	9.8 lb/1,000 ft^2
SoilGard	*Gliocladium virens* strain GL-21 (1×10^6 CFU/g)	Granular	4 oz/1,000 ft^2
Serenade	*Bacillus subtilis* strain QST 713 (1×10^9 CFU/g)	Liquid	192 oz/a

Treatments were arranged in a randomized complete block design and replicated four times. Sclerotia were preconditioned in moist sterile soil at 41°F for 6 weeks during spring of 2016. Treatments were applied at labeled rates, and the sclerotia were incubated at 60°F for 7 weeks. The numbers of germinating sclerotia and fruiting bodies (capitula) were noted for area under capitula production curve (AUCPC) calculations, and data were analyzed using ANOVA.

Results and Discussion

Isolation and screening of fungi and bacteria on *C. purpurea* sclerotia

The microflora isolated from the baited ergot sclerotia in Hermiston, OR and Madras, OR were a complex of fungi, including *Fusarium* species (most commonly *Fusarium avenaceum* and *F. incarnatum*), *Pythium* spp., *Alternaria* spp., *Epicoccum nigrum*, and zygomycetes. Isolated bacteria included members of the Enterobacteriaceae family, namely *Pantoea* spp. and *Erwinia* spp., as well as members of the *Pseudomonas* spp. complex. Interestingly, the genera and species of microflora isolated from baited sclerotia were similar between the Columbia Basin and central Oregon. Unfortunately, culture filtrates of the selected microorganisms did not inhibit ergot sclerotia germination. In addition, many of these organisms are potential plant pathogens, which would limit their use as biocontrol options for ergot.

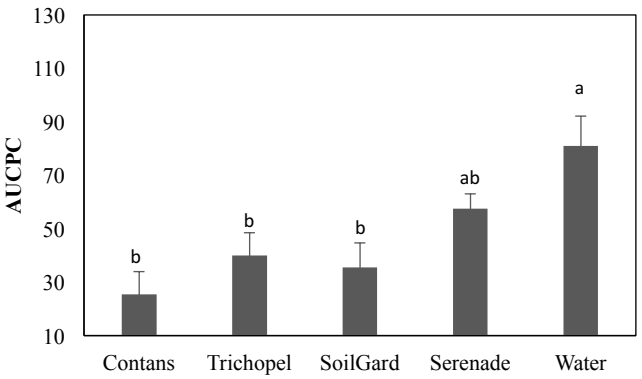

Figure 1. Mean area under capitula production curve (AUCPC) values in experimental petri plates containing ergot sclerotia treated with various biocontrol fungicides.

Evaluation of commercial biocontrol products against sclerotia germination

Laboratory assays resulted in significant reductions in AUCPC ($P = 0.0008$) after treatment with Contans, Trichopel, and SoilGard, compared to the water-treated control plates (Figure 1). Application of Contans reduced AUCPC values by 68.5%, compared to the control, while Trichopel and SoilGard reduced AUCPC values by 55.6 and 50.6%, respectively. Contans has been reported as an effective sclerotial mycoparasite in a variety of crops (Whipps et al., 2008). Antagonistic activities were previously reported for products containing *Trichoderma viride*, *Trichoderma harzianum*, and *Gliocladium virens* against sclerotia germination of *Claviceps fusiformis* (Mohan and Jeyarajan, 1990).

Conclusion

Diverse plant pathogenic and saprophytic microorganisms were found to be associated with *C. purpurea* sclerotia in commercial grass seed production fields. However, none of these was observed to be a mycoparasite of ergot. On the other hand, some commercial biocontrol products that are labeled for management of other sclerotia-producing fungi were found to reduce sclerotia germination in laboratory assays. Additional testing of these products is currently underway in field trials located at Hermiston Agricultural Research and Extension Center, Hermiston, OR.

References

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