SPATIAL PATTERNS OF *CLAVICEPS PURPUREA* IN KENTUCKY BLUEGRASS AND PERENNIAL RYEGRASS GROWN FOR SEED AND EFFECT OF SOIL-APPLIED FUNGICIDES ON GERMINATION OF ERGOT SCLEROTIA

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Introduction

Ergot, caused by the fungus *Claviceps purpurea*, is an important floral disease of grasses that significantly impacts Kentucky bluegrass (KBG) and perennial ryegrass (PRG) seed production in the Pacific Northwest (PNW). The fungus infects unfertilized ovaries and replaces seed with elongated black fungal bodies called sclerotia, which overwinter in the soil and germinate to produce airborne ascospores in the spring. In addition to yield losses from ergot, the repeated cleanings required to remove ergot sclerotia to achieve seed certification standards result in additional seed loss as well as extra costs in time and labor.

Although ergot is a persistent problem in PNW grass seed production, the incidence and intensity of ergot epidemics can vary regionally, locally, and from year to year. Several studies have investigated the occurrence and distribution of ergot in different production areas of Oregon, but little is known about the spatial patterns of ergot epidemics within large commercial fields. Understanding the spatial patterns of ergot in grass grown for seed can provide important information that can be used to improve ergot sampling and assessment, identify and target sources of inoculum, and develop more effective management tactics.

Germinating sclerotia of *C. purpurea* are considered to be the source of primary inoculum for ergot epidemics in grass seed fields. Although some sclerotia are removed with seed during harvest or with plant debris during the cutting and baling of straw, sclerotia are often dislodged from infected inflorescences and returned to the field during mechanical harvest. Sclerotia that remain in the field following harvest can overwinter and increase the amount of primary inoculum available for the next year, especially in perennial grass seed crops (Alderman *et al*. 1993).

Ergot control in grass seed production in the PNW has been improved in recent years, but multiple protective fungicide applications are required during anthesis. Some growers experience severe ergot issues even after four fungicide applications. A limited number of fungicides are currently available and new products or application strategies need to be evaluated. In addition to timing fungicides during anthesis, when flowers are susceptible to infection, it may be possible to apply fungicides to sclerotia in the field during the fall and/or as they begin to germinate in the spring before they release spores. Soil-applied fungicides may reduce sclerotia germination and spore production, limiting the production of primary inoculum and subsequent ergot infection (Hardison 1975).

The objectives of this study were to: 1) quantify and describe the spatial patterns of ergot epidemics in commercial KBG and PRG fields in Oregon and Washington; 2) quantify the number of sclerotia left in fields after harvest and post-harvest management operations; and 3) investigate the effectiveness of soil-applied fungicides at reducing the germination of ergot sclerotia *in vitro*. Together, this research should provide a better understanding of *C. purpurea* inoculum sources, the etiology and development of ergot epidemics in commercial fields, and additional options for the chemical control of ergot.

Materials and Methods

Fields

Three 125 acre commercial PRG fields (cultivars ‘Pavilion’, ‘Provocative’, and ‘Top Hat II’) near Hermiston, OR were included in the study. All three fields were in the first year of production, under center pivot irrigation, and subjected to similar cultural practices. Two commercial KBG fields located near Paterson, WA (R-1 and BH-6) and four commercial KBG fields located near LaGrande, OR were also included. Both fields near Paterson, WA were first-year, 125 acre fields (cultivar ‘Midnight’) under center pivot irrigation. Fields near LaGrande, OR were between 2 and 4 years old and of varying cultivars and acreages (Table 1). All fields near

LaGrande were under hand or wheel line irrigation except the field of cultivar 'Kelly', which was irrigated under a one-half center pivot.

Disease Assessment and Spatial Analyses

Fields were surveyed about one week prior to harvest. Sample points were located along wheel tracks and consisted of quadrats approximately 10 ft^2 in size spaced 30 to 100 ft apart. Quadrats were located 6.5 ft away from the wheel track and were mapped using a GPS unit. Inflorescences were arbitrarily collected from each quadrat for evaluation in the lab. The number of sclerotia was counted in each inflorescence to determine incidence and severity at each quadrat. Spatial autocorrelation and aggregation of disease severity were determined using Moran's I and the SADIE indices of aggregation, patch clusters, and gap clusters. Patch and gap clusters are defined as regions with relatively large or small counts in close proximity to each other, respectively, and the SADIE indices measure the degree to which each quadrat contributes to a patch or cluster. These statistics were used to determine if the intensity of ergot was evenly distributed throughout the field or if the disease tended to occur in patches, or foci.

Postharvest Collection and Quantification of Sclerotia

Samples of crop debris, soil, sclerotia, and seed were collected following harvest and residue management operations. Samples were collected from the three commercial PRG fields near Hermiston, OR and three of the commercial KBG fields near LaGrande, OR (cultivars 'Baron', 'Kelly' and 'Right') described in Objective 2. Postharvest residue was baled and removed from PRG fields, while KBG fields near La Grande were propane flamed. A commercial vacuum-sweeper, towed at approximately 1 mph, was used to collect samples from 20 to 24 plots per field. Each plot was approximately 6.5 ft wide and 16 or 32 ft long. Seed and sclerotia were separated from soil and plant residues using an air screen machine, indent cylinder, air column separator, and hand screens. A stereo microscope was used to identify and count sclerotia.

Effect of Soil-applied Fungicides on Sclerotia Germination

Fresh sclerotia from KBG and PRG were obtained from seed cleaning facilities in August. Sclerotia from both hosts were used since sclerotia from KBG are typically much smaller (≤ 1 mg) than those from PRG (4 to 70+ mg) and may respond differently to soil-applied fungicides. A total of four replicate plates, each containing 25 sclerotia, were used for each combination of sclerotia type and fungicide treatment. The experiment was arranged in a randomized complete block design. Treatments were intended to simulate a spring application, so sclerotia were preconditioned in moist sterile soil at 41°F for six weeks prior to fungicide treatments. Eight fungicides, including Endura (boscalid at 5.6 lb a.i./gallon), Botran 5F (dicloran at 5 lb a.i./gallon), Omega 500F (fluazinam at 4.2 lb a.i./gallon), Blocker 10G (PCNB at 75% a.i. w/w), Quilt Xcel (azoxystrobin+propiconazole at 2.2 lb a.i./gallon), Propulse (fluopyram+prothioconazole at 3.4 lb a.i./gallon), DPX-PZX74 (picoxystrobin+cyproconazole at 2.3 lb a.i./gallon), and Priaxor (pyraclostrobin+fluxapyroxad at 4.2 lb a.i./gallon), onion oil (2.4 and 0.24 gallon/acre), onion compost (24.5 gallon/acre), and a sterile water control were tested. Tween, a nonionic surfactant, was added to the onion oil and fluopyram+prothioconazole treatments (1 qt/100 gal) as recommended by the manufacturer. Treatments were applied directly to ergot sclerotia using a handheld sprayer calibrated to dispense 750 µl/plate, equivalent to 100 gallon/acre. Sclerotia were subsequently placed in a 60°F incubator for seven weeks. The number of germinating sclerotia and capitula (spore-producing fruiting bodies) were recorded weekly. Germination ratios were calculated by dividing the total number of germinated sclerotia for each experimental unit by the mean number of germinated sclerotia of the sterile water control treatment. A germination ratio < 1 indicates reduced germination compared with the mean germination ratio of the water-treated control. Data were subjected to ANOVA and treatments were compared using Dunnett's test to determine if any were significantly different than the control.

Results and Discussion

Disease Assessment and Spatial Analyses

A total of 1433 and 1613 quadrats were examined in the three PRG and six KBG fields, respectively. The percentage of quadrats containing at least one

inflorescence bearing sclerotia ranged from 59 to 90% in the PRG fields. The percentage of quadrats with sclerotia in KBG fields ranged between 0 and 49%. Sclerotia were not observed in field R-1, but 36% of quadrats contained at least one inflorescence exhibiting honeydew. The mean incidence of inflorescences bearing sclerotia ranged between 1.9 and 25.3%, while mean severity, or the mean number of ergot bodies per 10 to 30 inflorescences, ranged from 0.68 to 4.13. Results are summarized in Table 1.

Surveys and plots of disease intensity indicated that although ergot incidence was widespread within the fields, disease severity was not evenly distributed throughout (Fig. 1). Significant ($P \leq 0.002$) clustering of disease severity was observed in all three PRG fields and bluegrass field BH-6 using Moran's I and SADIE indices. Foci of high ergot severity in fields may be caused by secondary spread within and among neighboring plants via honeydew, focused sources of primary inoculum, or a combination of environmental and biological factors. In contrast, ergot was randomly distributed in bluegrass field of cultivar 'Baron', which was in its fourth year of production. Negative binomial regression analysis indicated that ergot severity in PRG fields H-3, H-7, and RC-2 significantly ($P < 0.006$) decreased with increasing distance from established, nearby PRG fields, and especially PRG fields located upwind. These results suggest that older PRG seed fields may be important sources of inoculum for neighboring first-year fields. However, the contribution of infected weeds, volunteers, or native plants to ergot epidemics also requires further investigation.

Postharvest Collection and Quantification of Sclerotia

Sclerotia remaining in the field post-harvest varied among the three PRG fields, with mean sclerotia/ft^2 at 0.41, 0.35 and 1.42 for fields H-3, H-7 and RC-2, respectively. In fields H-3 and H-7, 77% of plots had 5 or fewer sclerotia/10 ft^2. High sclerotia levels were found in the RC-2 field, with more than 10 sclerotia/10 ft^2 recovered from 50% of the sites and one site contributing over 70 sclerotia/10 ft^2. Post-harvest assessments of ergot from the KBG fields were not completed due to difficulties in identifying sclerotia among the burned surface residues.

Effect of Soil-applied Fungicides on Sclerotia Germination

A significant ($P \leq 0.05$) reduction in germination ratio was observed in KBG and PRG sclerotia treated with picoxystrobin+cyproconazole or azoxystrobin+propiconazole and in KBG sclerotia treated with pyraclostrobin+fluxapyroxad or fluopyram+prothioconazole compared to water-treated controls (Fig. 2). Reduced capitula production was also observed. Pyraclostrobin+fluxapyroxad, picoxystrobin+cyproconazole, and azoxystrobin+propiconazole delayed PRG sclerotia germination by at least 2 weeks. Pyraclostrobin+fluxapyroxad also delayed KBG sclerotia germination by two weeks, while picoxystrobin+cyproconazole and azoxystrobin+propiconazole prevented germination of KBG sclerotia for the entire 7-week period. These results indicate that soil-applied fungicides have the potential to reduce or delay the germination of overwintered ergot sclerotia, preventing the release of ascospores during anthesis and disrupting the ergot disease cycle. Future studies performed in the lab and in the field will focus on the most efficacious chemistries and the impacts of single and multiple fungicide applications on sclerotia germination and capitula development.

References

Alderman, S.C., Churchill, D.B., and Bilsland, D.M. 1993. A vacuum collection and seed separation technique for enumeration of sclerotia of *Claviceps purpurea* in perennial ryegrass fields. Plant Disease 77:1020-1022.

Hardison, J.R. 1975. Chemical prevention of ascocarp formation in *Claviceps purpurea*. Phytopathology 65:502-503.

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Table 1. The number of quadrats examined, ergot incidence and the mean incidence of infected inflorescences and ergot severity in three fields of perennial ryegrass and six fields of Kentucky bluegrass grown for seed.

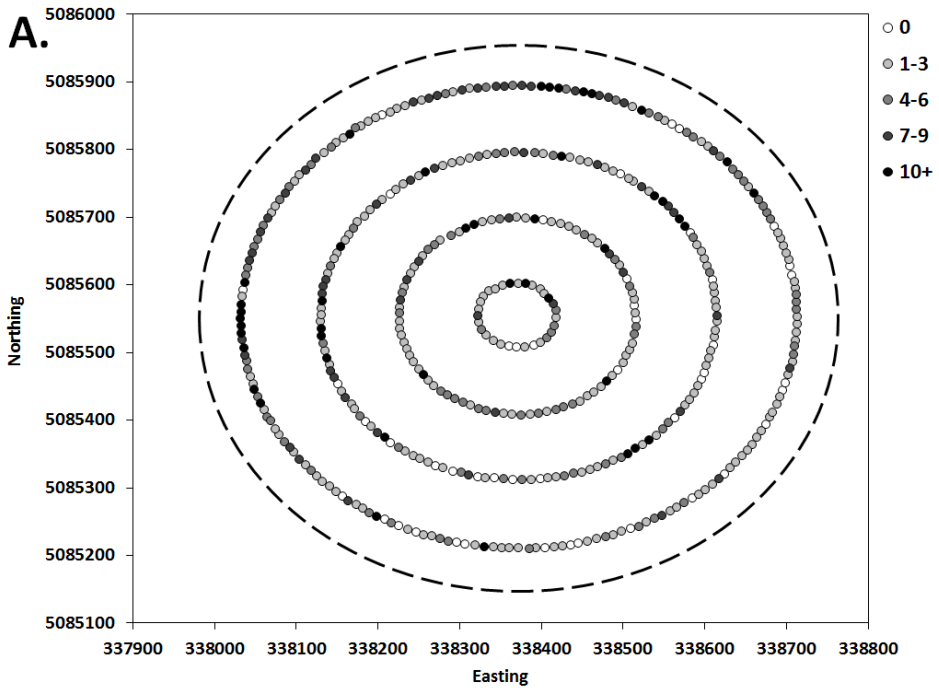
Grass	Field	Cultivar	Acres	Years cropped to grass seed	No. quadrats[a]	Quadrats with ergot (%)[b]	Mean incidence of infected inflorescences (%)[c]	Mean Severity[d]
Perennial ryegrass	H-7	Provocative	125	1	454	90	25.3	4.13
	H-3	Pavilion	125	1	492	84	20.5	2.86
	RC-2	Top Hat II	125	1	487	59	12.7	1.79
Kentucky bluegrass	BH-6	Midnight	125	1	589	29	1.9	0.68
	R-1	Midnight	125	1	483	0	0.0	0.00
	BR-20	Baron	32	4	115	49	23.3	0.38
	08-651	Kelly	65	4	185	< 1	< 0.1	< 0.01
	10-06	Wildhorse	40	2	139	0	0.0	0.00
	10-02	Right	77	2	102	0	0.0	0.00

[a] Quadrats were approximately 10 ft^2 in size and approximately 30 to 100 ft apart. Quadrats were located at approximately 6.5 ft outside of every other pivot wheel track. At least 10 inflorescences were collected from each quadrat.

[b] Percentage of quadrats with at least one inflorescence bearing sclerotia.

[c] Mean percentage of inflorescences bearing sclerotia per quadrat.

[d] Mean number of ergot bodies per quadrat.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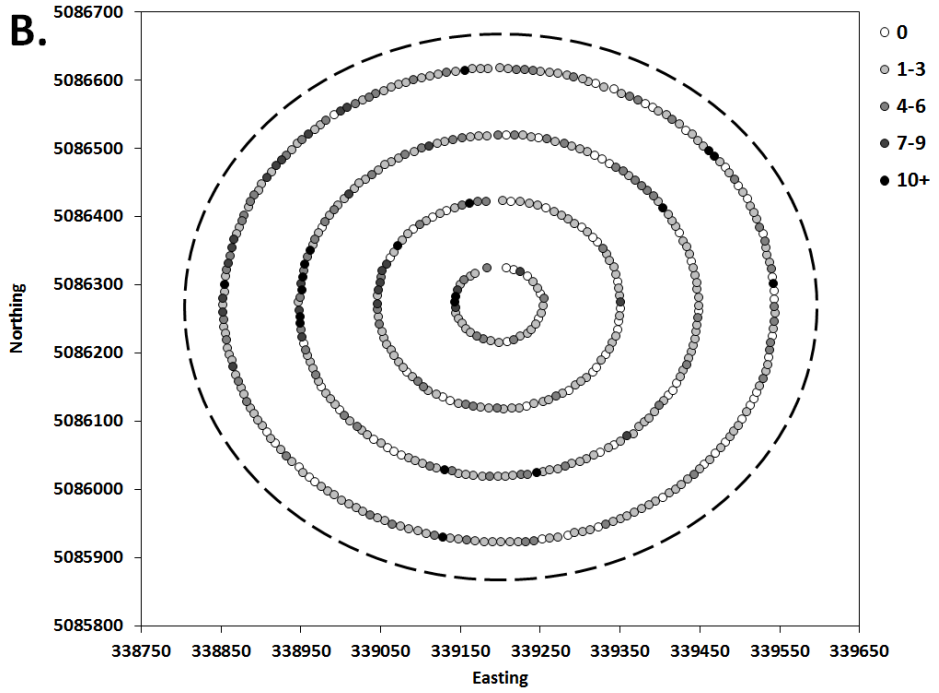
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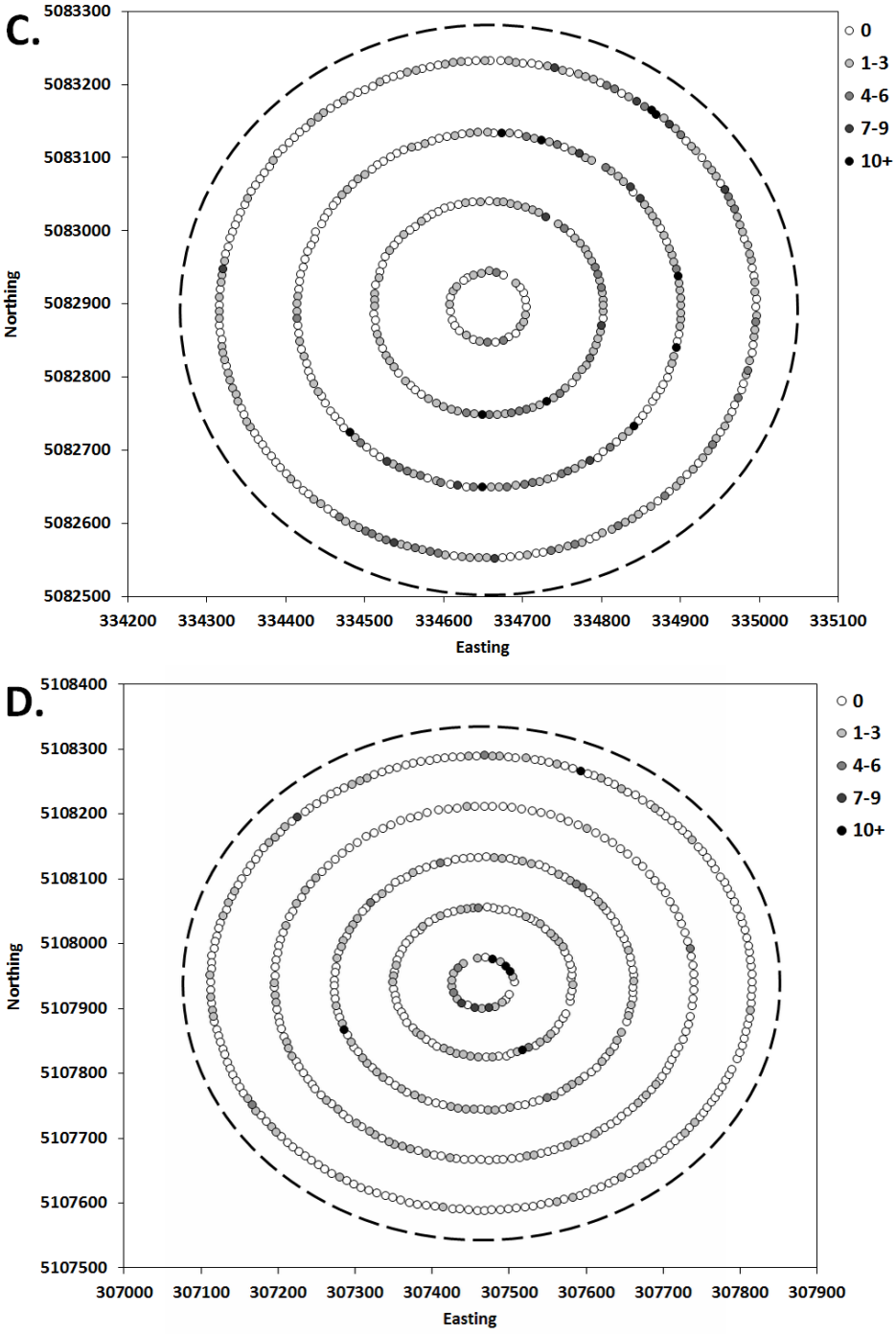

Fig. 1. Dot plots of ergot severity in perennial ryegrass fields A) H-7; B) H-3; C) RC-2; and D) Kentucky bluegrass field BH-6. Values represent the total number of sclerotia in ten inflorescences collected from sampled quadrats located 30 ft apart and following alternating center pivot wheel tracks. Field boundaries are indicated by dashed lines.

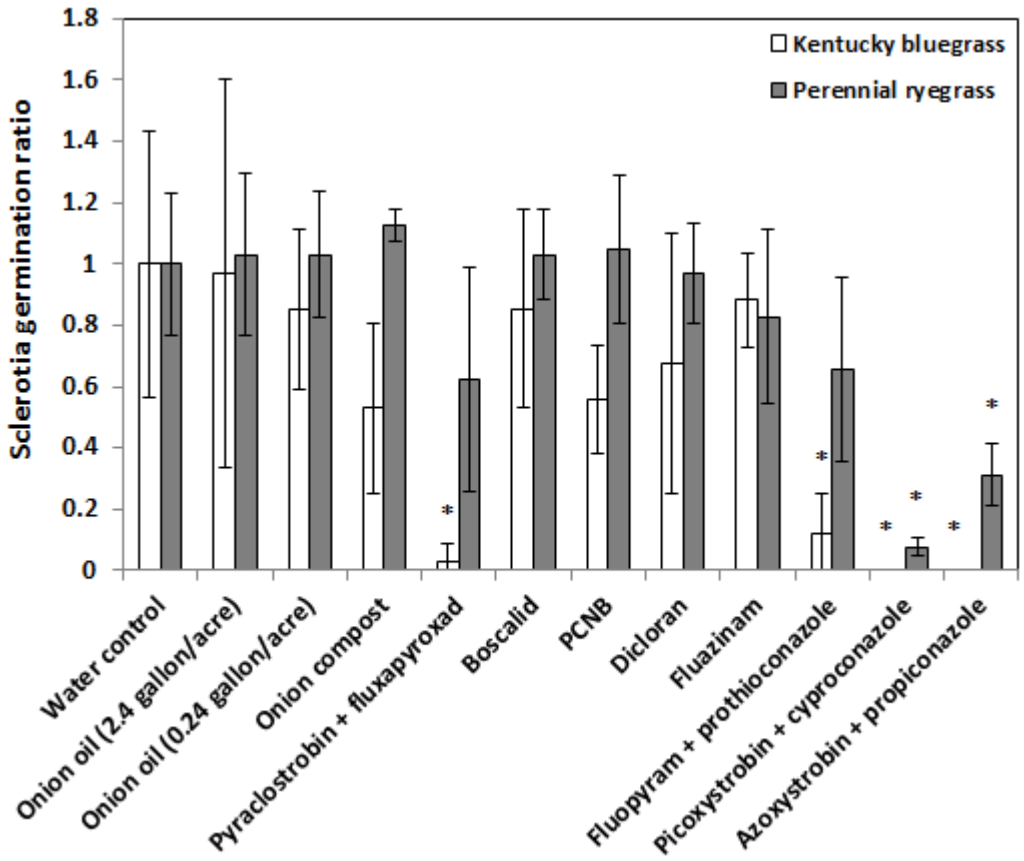

Fig. 2. Effect of onion oil, onion compost, and various fungicides on the germination of ergot sclerotia collected from Kentucky bluegrass and perennial ryegrass. Sclerotia germination ratio values were calculated by dividing the mean of each observation by the overall mean of the control treatments. Error bars represent standard deviations. Treatments labeled with an asterisk are significantly different than the water control using Dunnett's test at $P = 0.05$. Products used above are not registered for use in grass seed production and/or as soil applications. Always rely on the product label for complete details.