WASHINGTON TURFGRASS SEED COMMISSION Final REPORT
FOR 2016 PROJECTS

Project No.:_____

Title: Integrated Disease Management of Ergot in Kentucky Bluegrass

Personnel: Jeremiah Dung, Oregon State University (OSU), Madras, OR; Kenneth Frost, OSU, Hermiston, OR; Navneet Kaur, OSU, Hermiston, OR; Darrin Walenta, OSU, La Grande, OR; and Stephen Alderman, USDA-ARS, Corvallis, OR.

Reporting Period: November 2015-November 2016 (Objective 1b and 3a ongoing); November 2013-November 2016 (Objectives 1a, 2a and 2b completed/terminated)

Accomplishments:
Our research focus is to develop a comprehensive, multi-tactic IPM program for ergot in grass seed crops that incorporates chemical controls, biological controls, host plant resistance, and disease modeling. Soil-applied fluopyram + prothioconazole consistently reduced sclerotia germination over 4 years of study and soil applications of azoxystrobin, azoxystrobin + propiconazole, and picoxystrobin significantly reduced sclerotia germination in 2 of 4 years. Applications of fluopyram + prothioconazole during flowering exhibited similar performance to the industry standard in two years of study. The commercial biocontrol products Contans, SoilGard, and Trichopel reduced sclerotia germination in a lab assay. Greenhouse experiments suggest that cultivars of Kentucky bluegrass (KBG) and perennial ryegrass (PRG) respond differently to infection by different *Claviceps* species occurring in PNW grass seed production areas. Earlier flowering was negatively correlated with greater ergot incidence and severity in PRG plots in the Columbia Basin in 3 of 3 years. Predictive models have been developed using spore trap data collected from PRG fields in the Columbia Basin. These models were 72 to 88% accurate between 2013 and 2015 and up to 80% accurate when validated against historical data. A degree day period between 414 and 727 accounted for the occurrence of 94% of ascospores between 2013 and 2015 and was validated against data collected in 2016. As part of our research and outreach, we published the weekly Ergot Alert Newsletter during the grass seed growing season. The Ergot Alert Newsletter was sent to over 400 growers and stakeholders and included spore counts from statewide trapping efforts, cultivar phenology evaluations from three production areas of Oregon, and regionally-focused disease management recommendations. Together, this research contributes towards the development of comprehensive IPM strategies for ergot in grass seed crops of Oregon.

Results:
Objective 1) We hypothesized that fungicides and biocontrol products can reduce sclerotia germination when applied to soils, reducing the amount of airborne ascospores which are a major source of primary inoculum. Additionally, novel fungicide chemistries needed to be tested for their ability to protect flowers from infection during anthesis to reduce ergot incidence and severity when ascospores are present. Objective 1 was to evaluate new/unlabeled chemical

products and biological controls to protect flowers during anthesis and/or prevent the germination of ergot sclerotia.

Objective 1a) Field plots of KBG were established in August 2015 and infested with 100 sclerotia/plot. A total of 12 fungicides (Table 1) were applied in October (fall treatment), April (spring treatment), and/or in both months (fall + spring treatment). Treatments were replicated 4 times in 10 ft^2 plots. Sclerotia germination was assessed weekly from May-June. Significant reductions in area under capitula production curve (AUCPC) values ($P < 0.001$) were observed in some fungicide treatments compared to the water-treated control plots (Fig. 1). Fall + spring applications of azoxystrobin (Abound) reduced AUCPC values by 75% and fall applications of fluopyram+prothioconazole (Propulse) resulted in 72% reduction in AUCPC values compared to the untreated water control. These findings are consistent with results obtained in 2013-2015, where these two products were the found to be most effective at reducing sclerotia germination.

Objective 1b) Several newly released fungicides significantly reduced ergot infection when applied to PRG flowers during anthesis in 2015 field trials. Field plots (70 ft^2) were established to repeat the trial in 2016 and determine the reproducibility of results in different growing seasons. Pyraclostrobin + fluxapyroxad (Priaxor), benzovindiflupyr (Solatenol), penthiopyrad (Fontelis), and fluopyram + prothioconazole (Propulse) were applied using labeled rate during anthesis and compared to a non-treated control and a QuiltXcel (azoxystrobin + propiconazole) industry standard. Honeydew incidence and number of sclerotia were quantified to determine if new fungicides can reduce ergot incidence and severity. Applications of azoxystrobin + propiconazole (QuiltXcel) and fluopyram + prothioconazole (Propulse) during anthesis significantly ($P = 0.04$) reduced ergot honeydew (Fig. 2) compared to the untreated control. No differences in disease severity were observed.

Objective 1c) A lab assay was conducted to evaluate commercial biocontrol products against *Claviceps*. Treatments (Table 2) were applied to petri plates containing soil and sclerotia and replicated 4 times. The number of germinated sclerotia was compared to a non-treated control. Separate petri plate assays were conducted to identify and screen naturally-occurring fungi and bacteria on ergot sclerotia for use as potential biocontrol agents. Significant reductions in AUCPC ($P = 0.0008$) were observed after treatment with Contans, SoilGard, and Trichopel compared to the water-treated control plates (Fig. 3). These results suggest that field evaluations of potential biocontrol agents for ergot management may be warranted. The microflora isolated from the baited ergot sclerotia were a complex of plant pathogenic fungi including *Fusarium* species (most commonly *F. avenaceum* and *F. incarnatum*) *Pythium* spp., *Alternaria* spp., *Epicoccum nigrum*, and zygomycetes. Isolated bacteria included members of Enterobacteriaceae, namely *Pantoea* spp., *Erwinia* spp., and *Pseudomonas* spp. Microflora on baited sclerotia were similar between the Columbia Basin and central Oregon. Culture filtrates of these microorganisms did not inhibit ergot sclerotia germination.

Objective 2) The ergot fungus only infects unfertilized flowers, so ascospore production by the fungus must coincide with the flowering of susceptible grass hosts in order for infection to occur. In some years the timing of spore release and host anthesis does not coincide, resulting in little to no ergot. We hypothesized that cultivars with shortened and uniform flowering periods or cultivars with flowering times outside of peak ascospore production have the potential to escape

ergot infection. Objective 2 was to evaluate PRG and KBG cultivars for their potential to escape or resist ergot infection.

Objective 2a) PRG and KBG cultivars that consistently exhibit little to no ergot may either escape infection (i.e. avoiding inoculum through morphological or other mechanisms, see Objective 2b) or exhibit true resistance to infection (i.e. limiting growth and colonization of the pathogen during or after inoculum makes contact with the host). Twelve cultivars each of PRG and KBG were artificially inoculated to evaluate them for true resistance to *C. purpurea* and *C. humidiphila*. The experiment was conducted in the greenhouse and each treatment was replicated 3 times. Cultivars were evaluated for ergot incidence and severity after inoculation with either *Claviceps* species. Significant differences in disease incidence and severity ($P < 0.0001$) were observed among cultivars inoculated in the greenhouse (Table 3). Consistent with our 2014 results, *C. purpurea* exhibited significantly greater disease incidence and severity among PRG cultivars compared to *C. humidiphila*. *Claviceps purpurea* also caused greater incidence and severity in KBG compared to *C. humidiphila* but reactions varied among cultivars (Table 4). In general, Midnight II exhibited the greatest ergot severity (similar to field trials) and Blue Ghost, Bluechip, Gladstone, and PST-K4-7 exhibited the lowest ergot severity. These results suggest that differences exist in the ability of *C. purpurea* and *C. humidiphila* to infect PRG and KBG cultivars.

Objective 2b) Some grass cultivars may escape ergot infection by exhibiting shortened, more uniform flowering periods or by flowering when ascospores are not present. In 2015, several cultivars of PRG and KBG exhibited reduced ergot at harvest, and ergot severity was correlated with anthesis initiation date and duration. Field plots (120 ft^2) of PRG and KBG were established in August, each consisting of 12 cultivars replicated 4 times. Plots were infested with 200 sclerotia in October. Cultivars were evaluated for anthesis initiation and duration weekly and ergot incidence and severity at harvest. There was a significant difference between the time of flowering (e.g. anthesis initiation, anthesis termination and duration) among the PRG cultivars evaluated (Table 5). Consistent with our previous findings, PRG cultivar 'Quickstart II' exhibited the highest disease incidence among the cultivars evaluated based on the incidence of honeydew (Table 5). Earlier flowering was negatively correlated ($P = 0.009$; $r = -0.39$) with greater ergot incidence and severity in PRG plots in the Columbia Basin in 3 of 3 years, indicating that ergot was reduced in cultivars that initiated anthesis later in the year. Significant differences in ergot incidence and severity were observed among KBG cultivars at COARC, but disease levels and anthesis were not correlated (Table 6).

Objective 3) Spore trapping and weather data can be used to identify environmental factors which contribute to ergot germination and spore release and to develop a predictive model to forecast when inoculum is likely to be present. We hypothesized that an ergot phenology model can be used to inform growers if and when fungicides may be necessary and improve the timing of fungicide application to enhance ergot control. Objective 3 was to develop and validate a predictive model for ergot spore production.

From April-July, spore traps were placed in commercial fields in the Columbia Basin of Washington and Oregon, central Oregon, and northeast Oregon. Weather data, including minimum, maximum, and mean daily air and soil temperatures, mean daily relative humidity,

and mean daily dew point, were obtained from the field and established weather stations. Daily and cumulative air and soil degree days were calculated beginning January 1st and using upper and lower thresholds identified in previous studies. Predictive models developed using spore trap data from perennial ryegrass fields in the Columbia Basin were shown to be 72 to 88% accurate. Several variables including soil temperature and air temperature were identified that accounted for ascospore occurrence (Table 7). A degree day period between 414 and 727 accounted for the occurrence of 94% of ascospores between 2013 and 2015. The degree day model was tested against spore trap data in 2016 and accounted for 85 to 92% of ascospore occurrence, indicating that this degree day model is a useful predictor for ascospore production periods. Among commercial KBG fields, only one spore was captured in the Columbia Basin of WA and less than 25 spores were captured in Union County, OR. The lack of airborne spores at these sites between 2012 and 2016 suggests other inoculum sources (e.g. honeydew) may be more important sources of primary inoculum in certain areas of KBG seed production.

Publications (2016):

1. Dung, J.K.S., Alderman, S.C., Kaur, N., Walenta, D.L., Frost, K.E., and Hamm, P.B. 2016. Identification of weather factors related to *Claviceps purpurea* ascospore production and development and validation of predictive environmental favorability index models. Plant Disease (***accepted with revision***).
2. Dung, J.K.S., Alderman, S.C., Walenta, D.L., and Hamm, P.B. 2016. Spatial patterns of ergot and quantification of sclerotia in perennial ryegrass seed fields in eastern Oregon. Plant Disease 100(6):1110-1117. ***June 2016 Plant Disease Editor's Pick.***
3. Dung, J.K.S., Scott, J.C., Alderman, S.C., Kaur, N., Walenta, D.L., Frost, K.E., and Hamm, P.B. 2016. Development of a DNA-based protocol to detect airborne ergot spores in cool-season grass seed fields. Pages 31-34 in: 2015 Seed Production Research at Oregon State University USDA-ARS Cooperating. N. Anderson, A. Hulting, D. Walenta, M. Flowers, and C. Sullivan, eds. Oregon State University, Ext/CrS 152.
4. Dung, J.K.S., Scott, J.C., Alderman, S.C., Kaur, N., Walenta, D.L., Frost, K.E., and Hamm, P.B. 2016. Development and validation of a quantitative PCR assay for the detection of *Claviceps purpurea sensu lato* ascospores. Abstract. Phytopathology 106:S4.197.
5. Gilmore, B., Alderman, S., Knaus, B., Bassil, N., Martin, R., Dombrowski, J., and Dung, J.. 2016. Simple sequence repeat markers that identify *Claviceps* species and strains. Fungal Biology and Biotechnology 3:1 (doi:10.1186/s40694-016-0019-5)
6. Kaur, N., Alderman, S.C., Walenta, D.L., Frost, K.E., Dung, J.K.S., and Hamm, P.B. 2016. Evaluation of new fungicide chemistries and application strategies to reduce ergot in grass seed production systems. Pages 23-26 in: 2015 Seed Production Research at Oregon State University USDA-ARS Cooperating. N. Anderson, A. Hulting, D. Walenta, M. Flowers, and C. Sullivan, eds. Oregon State University, Ext/CrS 152.
7. Kaur, N., Cating, R., Dung, J.K.S., Alderman, S.C., Walenta, D.L., Hamm, P.B., and Frost, K.E. 2016. Rapid differentiation of *Claviceps* species occurring in Oregon and Washington using high resolution melting curve analysis. Abstract. Phytopathology 106:S4.113-114.
8. Kaur, N., Dung, J.K.S., Alderman, S.C., Walenta, D.L., Frost, K.E., and Hamm, P.B. 2016. Ergot escape potential of commercial cultivars of perennial ryegrass. Pages 27-30 in: 2015 Seed Production Research at Oregon State University USDA-ARS Cooperating. N. Anderson, A. Hulting, D. Walenta, M. Flowers, and C. Sullivan, eds. Oregon State University, Ext/CrS 152.

9. Walenta, D., Dung, J., Kaur, N., Alderman, S., Frost, K. and Hamm, P. 2016. Evaluating impact of a new information technology tool for ergot (*Claviceps purpurea*) management in Kentucky bluegrass and perennial ryegrass seed production systems of eastern Oregon. Proceedings of the 2016 National Association of County Agricultural Agents Western Region Annual Meeting and Professional Improvement Conference: 31-32
10. Walenta, D.L., Kaur, N., Alderman, S.C., Frost, K.E., Hamm, P.B., and Dung, J.K.S. 2016. Using information technology to advance integrated ergot disease management in perennial grass seed cropping systems. Pages 35-38 in: 2015 Seed Production Research at Oregon State University USDA-ARS Cooperating. N. Anderson, A. Hulting, D. Walenta, M. Flowers, and C. Sullivan, eds. Oregon State University, Ext/CrS 152.

Table 1. Fungicide treatments, trade name, manufacturer, rate, and timing of application in soil applied fungicide trial

Chemical	Product	Manufacturer	Rate (oz/acre)	FRAC group	Timing
Nontreated control	NA	NA	NA	NA	Fall + Spring
Pyraclostrobin + fluxapyroxad	Priaxor	BASF	6	7+11	Spring
Cyproconazole	Alto	Syngenta	5.5	3	Spring
Propiconazole	Tilt	Syngenta	8	3	Spring
Hydrogen dioxide + peroxyacetic acid	Oxidate 2.0	BioSafe Systems	32	NA	Spring
Hydrogen dioxide + peroxyacetic acid	Oxidate 2.0	BioSafe Systems	32	NA	Fall + Spring
Benzovindiflupyr	Solatenol	Syngenta	10.5	7	Spring
Penthiopyrad	Fontelis	DuPont	24	7	Spring
Penthiopyrad	Fontelis	DuPont	24	7	Fall
Fluopyram + prothioconazole	Propulse	Bayer CS	14	3+7	Fall
Fluopyram + prothioconazole	Propulse	Bayer CS	14	3+7	Spring
Fluopyram + prothioconazole	Propulse	Bayer CS	14	3+7	Fall + Spring
Azoxystrobin + propiconazole	Quilt Xcel	Syngenta	14	3+11	Fall
Azoxystrobin + propiconazole	Quilt Xcel	Syngenta	14	3+11	Spring
Azoxystrobin + propiconazole	Quilt Xcel	Syngenta	14	3+11	Fall + Spring
Pyraclostrobin	Headline	BASF	12	11	Fall
Pyraclostrobin	Headline	BASF	12	11	Spring
Pyraclostrobin	Headline	BASF	12	11	Fall + Spring
Picoxystrobin	Aproach	DuPont	18	11	Fall
Picoxystrobin	Aproach	DuPont	18	11	Spring
Picoxystrobin	Aproach	DuPont	18	11	Fall + Spring
Azoxystrobin	Abound	Syngenta	15.5	11	Fall
Azoxystrobin	Abound	Syngenta	15.5	11	Spring
Azoxystrobin	Abound	Syngenta	15.5	11	Fall + Spring

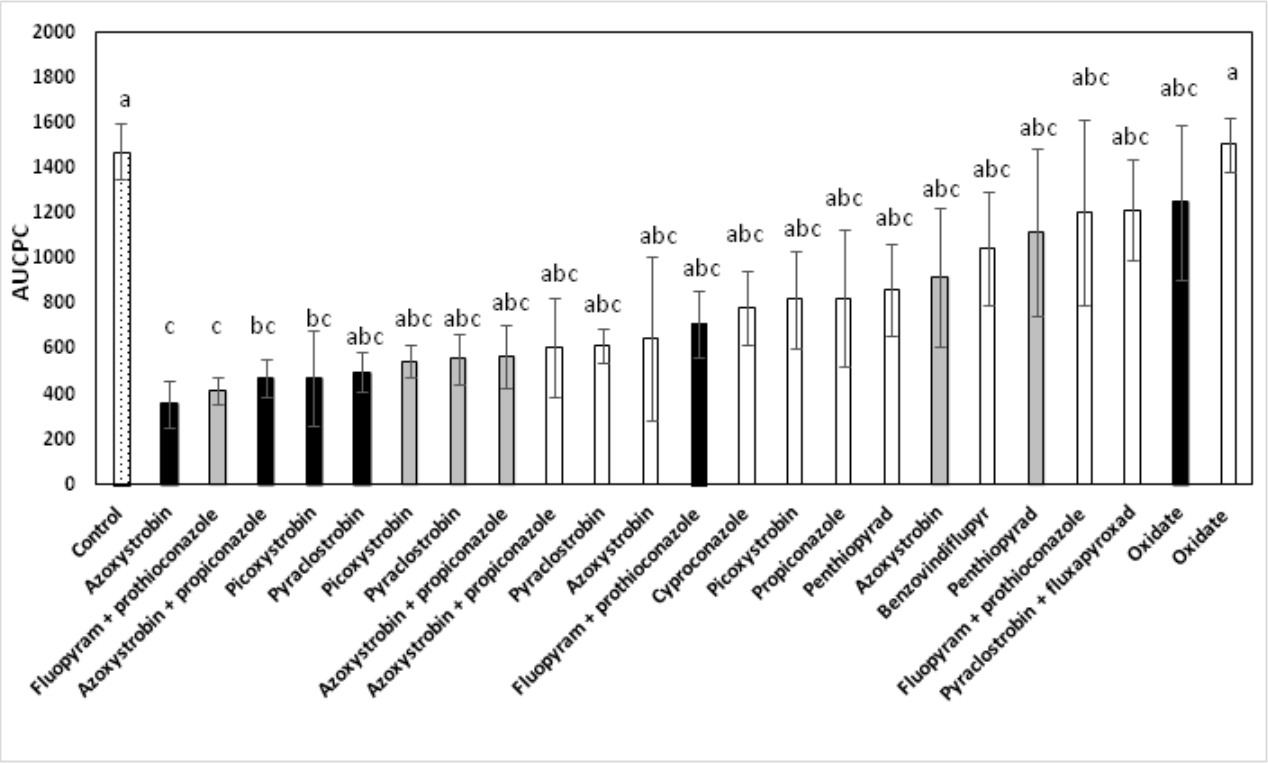

Fig. 1. Mean area under capitula production curve (AUCPC) values in experimental plots infested with ergot sclerotia from perennial ryegrass in October 2015 and treated with soil-applied fungicides in fall 2015 (gray bars), spring 2016 (white bars), or in both fall 2015 and spring 2016 (black bars). The dotted bar represents the water-treated control.

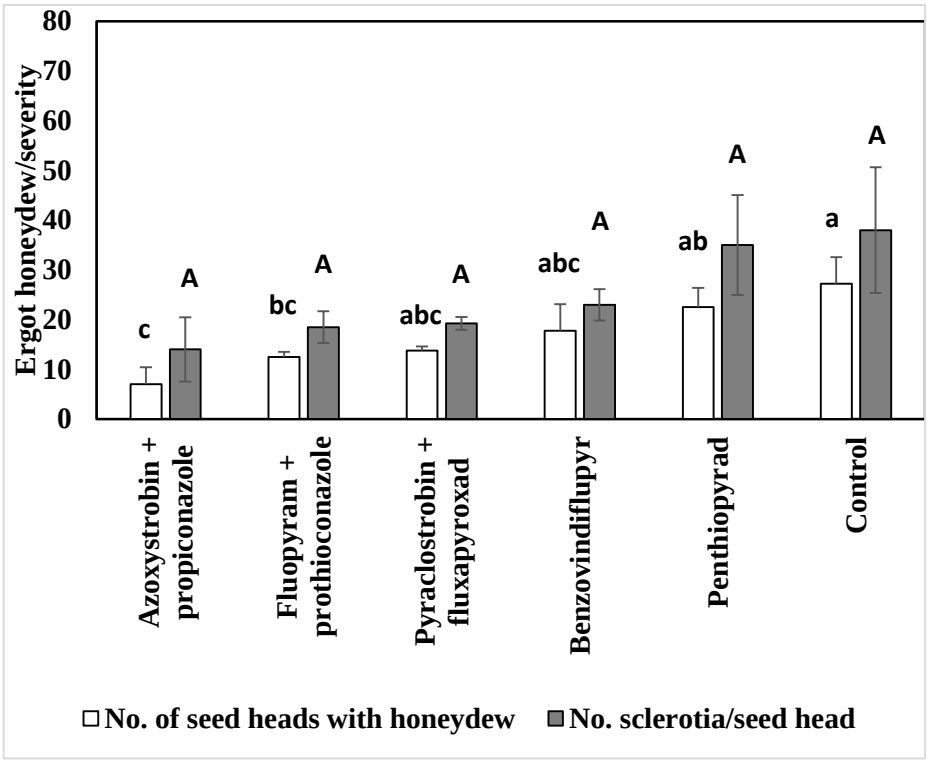

Fig. 2. Effect of fungicides on the number of perennial ryegrass seed heads with honeydew (blue bars) and the number of sclerotia in seed heads (red bars). Treatments with the same letters are not significantly different from each other.

Table 2. Trade names, active ingredients, formulations, and rates of commercial biocontrol products that were included in this study

Trade name	Active ingredient (concentration)	Formulation	Rate
Contans®	*Coniothyrium minitans* (1×10^9 CFU/g)	Wettable granules	2 kg/ha
Trichopel®	*Trichoderma harzianum* (1×10^6 CFU/g)	Granular	9.8 lbs/1000 ft^2
SoilGard®	*Gliocladium virens* strain GL-21 (1×10^6 CFU/g)	Granular	4 oz./1000 ft^2
Serenade® Soil	*Bacillus subtilis* strain QST 713 (1×10^9 CFU/g)	Liquid	192 oz. /acre

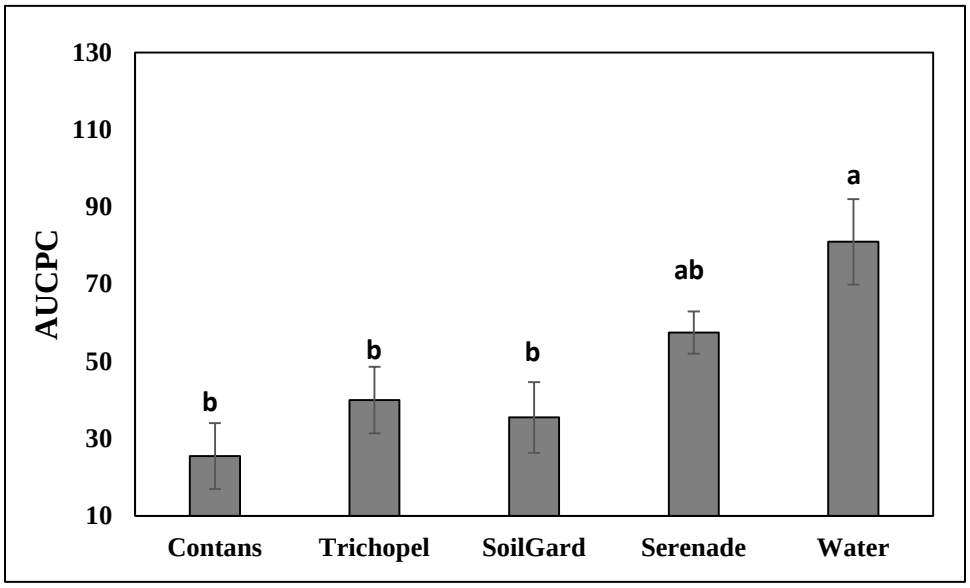

Fig 3. Mean area under capitula production curve (AUCPC) values in experimental petri plates containing ergot sclerotia treated with various bio rational fungicides.

Table 3. Incidence (number of perennial ryegrass seed heads with honeydew) of ergot infection and severity (mean number of sclerotia/seed head) after inoculating 12 perennial ryegrass (PRG) cultivars with a mix of *C. purpurea* and *C. humidiphila* isolates

	C. purpurea		***C. humidiphila***	
PRG cultivar	**Incidence (%)**	**Severity**	**Incidence (%)**	**Severity**
Applaud II	15.3a	21.0a	0.6ef	H[z]
1G2	15.3a	20.0a	2.0cdef	H
Integra II	3.3cdef	H	0.3f	0.3d
PST-2M20	2.0cdef	H	7.3bced	2.0d
Silver Dollar	6.3cdef	H	0.3f	H
Quickstart II	14.0ab	14.3ab	3.3cdef	4.0cd
Top Hat 2	7.3bcde	H	1.0def	H
Derby Extreme	8.0bc	H	1.0def	H
Esquire	7.6bcd	H	2.6cdef	H
Fiesta 4	8.0bc	14.3ab	1.0def	H
SR 4600	5.0cdef	H	4.3cdef	H
Karma	6.7cdef	11.3bc	5.0cdef	4.3cd
***P*-value**	**<0.0001**	**<0.05**	**<0.05**	**<0.05**

[z] H = only honeydew observed.

Table 4. Incidence (number of Kentucky bluegrass seed heads with honeydew) of ergot infection and severity (mean number of sclerotia/seed head) after inoculating 13 Kentucky bluegrass (KBG) cultivars with a mix of *C. purpurea* and *C. humidiphila* isolates

	C. purpurea		***C. humidiphila***	
KBG cultivar	**Incidence (%)**	**Severity**	**Incidence (%)**	**Severity**
Blue Ghost	0.0	0.0 b	4.2	0.3
Gateway	17.8	1.0 ab	17.8	0.7
Shamrock	0.0	0.0 b	11.1	0.7
Bluechip	11.1	0.3 ab	0.0	0.0
Gladstone	0.0	0.0 b	6.7	0.3
Nuglade	41.7	1.3 ab	25.0	1.0
PST-K4-7	2.6	0.3 ab	0.0	0.0
Fielder	4.2	0.3 ab	20.6	1.3
Midnight II	44.9	3.3 a	19.0	1.0
Jumpstart	33.3	1.3 ab	8.3	0.7
Right	4.8	0.3 ab	3.3	0.3
DB-1013	9.5	0.7 ab	21.7	1.0
Merit	26.7	1.3 ab	5.6	0.3
***P*-value**	**0.31**	**0.04**	**0.66**	**0.75**

Table. 5. Anthesis timing, total spores during anthesis, ergot incidence (number of seed heads with honeydew), and ergot severity (number of sclerotia/seed head) of eleven perennial ryegrass cultivars grown in infested field plots

Cultivar	Anthesis initiation	Anthesis end	Anthesis duration	Total spores during anthesis	Incidence (%)	Severity
Applaud II	125.0 b	153.5 c	28.5 ab	243.0 a	17.5 ab	32.8
1G2	136.0 b	159.0 abc	23.0 b	85.0 b	3.0 b	10.8
Integra II	135.5 a	157.3 bc	21.8 b	90.5 b	17.5 ab	34.3
PST-2M20	134.3 a	161.5 ab	27.3 ab	120.5 b	11.0 b	26.3
Silver Dollar	124.0 b	159.8 abc	35.8 a	251.0 a	17.0 ab	24.3
Quickstart II	123.8 b	159.8 abc	36.0 a	251.0 a	45.8 a	55.8
Top Hat 2	135.5 a	160.5 abc	25.0 b	119.0 b	4.8 b	9.3
Derby Extreme	136.5 a	165.0 a	28.5 ab	75.0 b	13.3 b	20.8
Fiesta 4	126.6 b	154.6 bc	28.0 ab	242.7 a	16.7 ab	19.5
SR 4600	133.3 a	159.3 abc	26.0 ab	123.5 b	13.5 b	21.8
Karma	137.3 a	160.3 abc	23.0 b	69.0 b	5.0 b	8.8
***P*-value**	**<0.0001**	**0.0019**	**0.0004**	**<0.0001**	**0.0050**	**NS**

Table 6. Anthesis timing, total spores during anthesis, ergot incidence (number of seed heads with honeydew), and ergot severity (number of sclerotia/seed head) of eleven Kentucky bluegrass cultivars grown in infested field plots

Cultivar	Anthesis initiation	Anthesis end	Anthesis duration	Total spores during anthesis	Incidence (%)	Severity
Blue Ghost	138.5 ab	155.5 ab	17.0 a	1269.0	26.8 d	81.8 b
DB-1013	133.0 a	157.3 ab	24.3 b	1582.8	17.3 c	57.0 ab
Fielder	133.0 a	157.3 ab	24.3 b	1582.8	7.3 ab	15.8 a
Gateway	144.0 bc	159.0 b	15.0 a	1475.0	18.0 c	38.3 ab
Gladstone	138.5 ab	157.3 ab	18.8 ab	1442.3	14.0 bc	38.8 ab
Jumpstart	138.5 ab	153.8 ab	15.3 b	1095.8	11.3 abc	25.8 ab
Merit	142.6 b	158.1 b	15.5 b	1423.5	10.9 abc	17.1 a
Midnight II	141.3 ab	159.0 b	17.8 ab	1545.3	31.0 d	153.8 c
PST-K4-7	144.0 b	159.0 b	15.0 b	1475.0	3.3 a	6.8 a
Right	135.8 ab	152.0 a	16.3 b	992.8	11.8 abc	37.3 ab
Shamrock	133.0 a	153.8 ab	20.8 ab	1236.3	11.5 abc	19.3 ab
***P*-value**	**0.0001**	**0.0032**	**0.0241**	**0.0518**	**< 0.0001**	**< 0.0001**

Table 7. Lower and upper thresholds and median and mean values for weather variables on days when at least one *Claviceps purpurea* ascospore was observed between 2013 and 2015 in Umatilla Co., Oregon and the percentage of total ascospores trapped when variables were between the lower and upper thresholds[y]

Variable	Lower threshold	Median	Upper threshold	Mean	Ascospores trapped (% total)
Minimum air T (F°)	44.2	48.9	54.3	49.3	67.6
Maximum air T (F°)	71.1	77.4	83.5	77.4	52.6
Mean air T (F°)	58.3	62.6	68.4	63.3	59.2
Air DD[z]	9.0	13.1	17.5	13.1	50.6
Air CDD[z]	414.2	543.8	726.7	573.8	94.0
Minimum soil T (F°)	61.2	64.4	68.7	64.6	75.4
Maximum soil T (F°)	67.3	73.6	79.0	73.4	34.7
Mean soil T (F°)	64.2	68.7	72.7	68.7	59.5
Soil DD[z]	14.6	19.1	22.9	18.5	51.3
Soil CDD[z]	386.3	577.3	841.9	626.8	47.5
Mean relative humidity (%)	44.6	48.7	54.4	50.5	42.0
Mean dew point (F°)	38.7	42.6	46.8	42.4	64.0

[y] Weather data were collected from the Oregon AgriMet HRMO weather station, which was located at the Hermiston Agricultural Research and Extension Center in Umatilla Co., Oregon and approximately 3.0 to 19.4 km from the field sites.

[z] Air and soil degree days (DD) and cumulative degree days (CDD) were calculated using the single-sine method with upper and lower thresholds of 77 and 50°F, respectively. CDD were calculated beginning on January 1.