Report of Accomplishments from Research Funded by the Washington Turfgrass Seed Commission (2014):

Title: Controlling Ergot in Kentucky Bluegrass

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Abstract:

Ergot is a major seed replacement disease of perennial ryegrass (PRG) and Kentucky bluegrass (KBG) grown for seed in the U.S. Pacific Northwest. The disease causes yield loss, reduced seed vigor, and difficulties during harvest, cleaning, and seed lot certification. The objectives of this research were to: 1) investigate new fungicide strategies for ergot control; 2) establish the basic parameters for a predictive model based on crop phenology and environmental factors which favor ergot development that will provide growers with a decision aid for ergot control; 3) evaluate the potential for KBG and PRG cultivars to resist or escape ergot infection; and 4) quantify the spatial and temporal dynamics of ergot spread from a point source of honeydew inoculum to determine the relative importance of secondary spread. Fall and fall+spring (dual) applications of Propulse reduced ergot germination by 76 and 58%, respectively. Dual applications of picoxystrobin + cyproconazole and a spring application of Tilt reduced ergot germination by at least 35%. Environmental variables based on soil temperatures provided the most accurate predictions of spore events and the models developed could predict the occurrence of ≥10 spores with 86 to 96% accuracy. Two PRG varieties ('PST-2M20' and 'Derby Extreme') exhibited less honeydew production. In microplot studies, greater disease incidence occurred in the field plots located closer to a point source of honeydew inoculum, indicating that secondary spread via honeydew could occur through insect or water movement. Preliminary studies indicated a strong association between insect populations and ergot severity in PRG fields, suggesting that insects may play a significant role in disease dispersal. Further studies on the transmission of ergot via insects and their ability to vector this disease are needed.

Introduction:

Grass seed is an important crop to the Columbia Basin region of Washington and Oregon. In 2011, Kentucky bluegrass (KBG) ranked 32nd of all agricultural crops in Washington and contributed $13,260,000 to the state economy. Perennial ryegrass (PRG) and other important grass seed crops are also grown in Washington. Much of the seed crop acreage is located in irrigated regions of south-central and south-east Washington. In Oregon, another $18,849,000 was generated from Kentucky bluegrass grown on about 12,600 acres in 2012; while in the same year production of PRG occurred on over 105,000 acres and was valued at over $111,000,000.

Ergot, caused by the fungal pathogen *Claviceps purpurea*, is a floral disease of grasses and a persistent problem in irrigated grass seed production. The fungus infects the unfertilized flowers of grasses and grains and transforms seed into dormant resting structures (sclerotia), which overwinter and produce primary inoculum (ascospores) the following season. Ergot is a seed replacement disease and directly reduces yield. In addition, infested grass seed lots must be cleaned to remove ergot, which costs the industry additional time, labor, and money. Repeated cleanings of highly infested seed lots ultimately results in lost seed during the cleaning process. Seed lots that cannot be adequately cleaned can be rejected for certification. Due to the toxicity of alkaloids contained in the sclerotia, contaminated screenings cannot be pelletized and used for livestock feed, generating a waste product that has to be dealt with; bales of grass straw contaminated with sclerotia are also an issue.

Ascospore production by the fungus typically coincides with flowering of the host. Varieties that have shortened and uniform flowering periods, or varieties with flowering times outside of peak ascospore production, have the potential to escape ergot infection. In some years the timing of spore release and host anthesis does not coincide, resulting in little to no ergot. Environmental conditions can contribute to this lack of "timing" between host anthesis and pathogen spore production. Spore trap 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 determine when inoculum is present. An ergot phenology model can be implemented in an IPM approach to identify if and when fungicides are necessary, improve the timing of their application, and provide growers with a decision aide for ergot control. Extensive spore trapping and weather data collection was performed in the 2012 and 2013 seasons, but more data are needed.

In addition to ascospores, asexual spores (conidia) are produced in large numbers and mix with plant sap exuded from infected ovaries, which is called honeydew. Honeydew can serve as secondary inoculum and spread ergot if splash- or insect-dispersed to uninfected flowers within a season. However, the contribution of honeydew to ergot spread in grass seed production is not known.

Although cultural management techniques can be effective at preventing ergot, the disease remains a major problem in the Columbia Basin. Growers often make multiple fungicide applications in an effort to prevent and control the disease, reportedly spending $60-75/ acre for unsatisfactory control. An additional $75/acre in good seed is lost during the repeated cleanings required to remove ergot. Due to the minor use status of grass seed pesticides, agricultural chemical manufacturers do not devote resources toward registration of fungicides for isolated production areas such as the Columbia Basin. As a result, only three fungicides are currently labeled to control ergot in grass seed crops. A reduction in the number of fungicide applications for ergot, while maintaining adequate ergot control, would provide a significant savings in fungicide application costs. Research conducted in 2012 and 2013 suggests under laboratory and field conditions suggest that soil-applied fungicides can reduce ergot sclerotia germination. The

potential of soil-applied fungicides to reduce sclerotia germination will be further investigated using single and dual applications of fungicides that demonstrated efficacy in previous studies.

Objectives:

- **Objective 1.** Assess the efficacy of soil-applied fungicides to reduce sclerotia germination.
- **Objective 2.** Establish the basic parameters for a predictive model based on crop phenology and environmental factors which favor ergot development that will provide growers with a decision aid for ergot control.
- **Objective 3.** Evaluate PRG and KBG varieties for the potential to escape or resist ergot infection.
- **Objective 4.** Quantify the spatial and temporal dynamics of ergot spread from a point source of honeydew inoculum.

Procedures:

- **Objective 1.** Soil-applied fungicide trials were conducted in replicated plots located at HAREC. A 0.10 acre plot was planted with KBG cultivar 'Midnight' in September 2013. Rows were divided into plots 3.3 ft long and spaced 3.3 ft apart. Plots were infested with sclerotia from KBG (100 mg, equivalent to approximately 100 sclerotia) and PRG (100 count) in October 2013. The fungicide treatments included the following chemistries that were effective in petri plate assays previously conducted in the lab: Abound (azoxystrobin; Syngenta), Alto (cyproconazole; Syngenta), Headline (pyraclostrobin; BASF), Luna (fluopyram; Bayer CS), Piraxor (pyraclostrobin + fluxapyroxad; BASF), Proline (prothioconazole; Bayer CS), Propulse (fluopyram + prothioconazole; Bayer CS), QuiltXcel (azoxystrobin + propiconazole; Syngenta), Tilt (propiconazole; Syngenta), picoxystrobin (DuPont), and picoxystrobin + cyproconazole (DuPont). Treatments were applied to plots in the fall (October 2013) and/or spring (April 2014) for a total of 24 treatment combinations. Each treatment combination was replicated four times and treatments were arranged in a randomized complete block design. All treatments were applied using a backpack sprayer in a total volume equivalent to approximately 100 gal/acre of water. The number of germinating capitula in each treatment was counted every 6 days and counts were converted to area under capitula production curves (AUCPC). The mean and maximum number of capitula observed for each treatment during the course of the experiment was also calculated. Data were analyzed using analysis of variance (ANOVA)
- **Objective 2.** Burkard 7-day volumetric spore traps were used to monitor the number of *C. purpurea* ascospores and grass pollen in the air over time in two commercial KBG fields located in Benton County, WA, two commercial KBG fields located in Union County, OR, one commercial PRG field located in Umatilla County, OR, and in the PRG variety trial located at HAREC described in Objective 3 below. Data loggers were placed in each field and recorded air temperature, soil temperature, relative humidity, and dew point on an hourly basis. Weather data was also obtained from weather stations located near the spore traps. Spore trap samples were stained and the numbers of spores were counted. Correlation and regression analyses were used to identify environmental variables that are associated with spore production for use in a predictive model.
- **Objective 3.** Replicated (4) plots of 12 PRG varieties were planted in September 2013.

Each plot was 30 ft. long and consisted of 7 rows of plants. Each plot was infested in October 2013 with 200 sclerotia collected from PRG lots harvested in 2013. A Burkard spore trap was placed near the plots to determine the timing of ascospore release. Weather data was recorded from the weather station located near the plots. The timing and duration of anthesis for each variety was recorded to determine the potential for disease escape by each cultivar. Once honeydew production initiated, disease incidence was recorded for two weeks based on the percentage of infected flowers out of 10 flower spikes collected randomly from each plot. Upon maturity, disease incidence was calculated based on number of seed heads containing ergot sclerotia out of 40 randomly collected seed heads. Disease severity was calculated based on the number of sclerotia present in each infected seed head. Seed heads were swathed and harvested to determine seed yield and the number of sclerotia per 100 g sample in each treatment. Data on anthesis dates and duration was analyzed using ANOVA and multiple comparisons were made using Tukey's test.

A greenhouse study was also conducted at COARC using artificial inoculation methods to determine if any PRG cultivars exhibited true resistance to ergot isolates from PRG or KBG. The 12 PRG varieties used in the field study described above were grown in a greenhouse until anthesis initiation and plants were inoculated with ergot conidia produced in liquid culture. Ergot treatments included ergot conidia from a mixture of 3 isolates obtained from PRG (10^6 conidia/ml), a mixture of 3 isolates obtained from KBG (10^6 conidia/ml), or sterile distilled water. Flowers were sprayed every 2 to 3 days until runoff. Each treatment combination was replicated 3 times. The number of sclerotia was determined for each plant at the conclusion of anthesis.

- **Objective 4.** A demonstration plot of PRG (60 ft by 30 ft) was planted in an isolated area of HAREC to avoid ascospore inoculum from infested plots used in other experiments. Eight microplots 6.5 ft by 6.5 ft in size were established. Four plots were approximately 3.2 ft from a point source of honeydew inoculum and the remaining four plots were plots were approximately 9.8 ft from the inoculum source. PRG plants were infected in the greenhouse and used as a source of honeydew inoculum. Infected plants were placed in the center of each plot at the beginning of anthesis. Ergot infection and spread in the micro-plots was monitored during and after anthesis to determine the rate of ergot spread from the source of honeydew in PRG plots. Data was analyzed using chi-square goodness of fit analyses.

  A separate, additional study was conducted to determine the potential for secondary spread of ergot via insects. The sugary matrix of honeydew can serve as a food resource for insects such as flies, beetles, wasps, and piercing and sucking insects that land or feed on the flowers and thereby facilitate the insect-mediated secondary dispersal of the ergot pathogen. This study was designed to monitor insect abundance in four commercial KBG and PRG fields each in the Columbia Basin region of Washington and Oregon from May to June 2014. The sampling techniques (Fig. 1) included universal black light traps, delta traps, yellow sticky cards, and modified sweep netting to avoid any direct contamination of honeydew during collection. Insects were sorted, counted and stored at −20ºC until microscopic examination for the presence of fungal spores. Ergot presence in or on insects was confirmed using a high-fidelity polymerase chain reaction developed in this study. Ergot incidence in the commercial fields was calculated based on the number of

infected seed heads out of 100 seed heads collected from each quadrant of field sampled. Correlations between ergot incidence and insect abundance were calculated.

Accomplishments:

- **Objective 1.** Similar to the first trial, ergot germination not observed in any of the field plots infested with ergot sclerotia collected from KBG. However, germination capitula were observed in all plots infested with ergot sclerotia collected from PRG. In contrast to the first year of the trial, significant reductions in sclerotia germination were not observed compared to the water-treated control plots (Fig. 2). However, dual and fall applications of Propulse reduced AUCPC values by 76 and 58%, respectively. Dual applications of picoxystrobin + cyproconazole and a spring application of Tilt reduced AUCPC values by at least 35%.
- **Objective 2.** Only 4 spores were detected in the two KBG fields in Benton County, WA. A total of 47 and 82 spores were captured in the two fields in Union County, OR between April 30 and June 22. In Umatilla County, OR, over 8,100 spores were captured in the commercial PRG field between April 30 and June 24 and over 1,100 spores were captured in the HAREC PRG variety plots between April 26 and June 13. The first occurrence of spores occurred at the two sites in Umatilla County, OR when accumulated air degree days were between 154 and 168 and accumulated soil degree days were between 135 and 161.

  The small number of spores observed in KBG fields hindered the ability to develop a model for spore production; however, significant progress was made in the development of a spore prediction model for ergot in PRG fields. Significant correlations ($P < 0.05$) were observed between spore counts and the following environmental variables collected from the HAREC weather station: minimum ($r = 0.25$), maximum ($r = 0.26$), and mean ($r = 0.29$) air temperatures, minimum ($r = 0.45$), maximum ($r = 0.24$), and mean ($r = 0.33$) soil temperatures, daily air ($r = 0.24$) and soil ($r = 0.38$) degree days, mean dew point ($r = 0.30$), and evapotranspiration ($r = 0.18$). Precipitation was not a significant factor in this study ($P > 0.67$), likely due to the regular irrigation that is required to grow grass seed crops in the semi-arid Columbia Basin.

  Local regression identified several trends that were used to identify upper and lower threshold values of environmental factors significantly correlated with spore production in PRG fields. The environmental factors used to predict ergot ascospore occurrence in this study included minimum daily air temperature between 41 and 54°F, maximum daily soil temperature between 59 and 72°F, minimum and mean daily soil temperatures between 57 and 70°F, daily soil degree days between 11 and 20, and mean daily dew point between 37 and 50°F. When used to predict the appearance of at least one spore, all environmental variables except minimum air temperature were significant ($P < 0.05$) predictors and ranged in accuracy from 60 to 83% (Table 1). All environmental variables were significant when used to predict the occurrence of at least 10 spores per day (64 to 90% accuracy) or at least 100 spores per day (60 to 89% accuracy) (Table 1). Environmental variables based on soil temperatures provided the most accurate predictions of spore events.

  A cumulative environmental favorability index (EFI) model was developed that included maximum soil temperature, minimum soil temperature, daily soil degree days, and mean dew point thresholds listed above. A cumulative EFI value of 2 correctly

predicted the occurrence of at least one spore with an accuracy of 82% and correctly predicted the occurrence of at least ten spores with an accuracy of 86% (Table 2). A cumulative EFI value of 3 predicted the occurrence of at least 100 spores with an accuracy of 91% (Table 2). These results suggest that predictive models can be a useful tool to predict ergot ascospore production in the Columbia Basin.

- **Objective 3.** The data on anthesis dates indicated that there is a significant difference between the time of flowering and anthesis duration among the cultivars evaluated (Table 3). The variety 'Quickstart II' initiated flowering significantly earlier in the season compared to other varieties and the anthesis period was significantly longer (Table 3). The flowering period of Quickstart II coincided with the ascospore production period (Fig. 3), resulting in the highest disease incidence among the varieties evaluated (Fig. 4). Two varieties 'PST-2M20' and 'Derby Extreme' exhibited significantly less disease incidence (P <0.0001) based on the incidence of honeydew (Fig. 4). However, significant differences were not observed for disease incidence and severity based on the number of sclerotia present (data not shown).

  Significant differences in ergot infection among cultivars were not observed in the greenhouse study (P > 0.05). However, PRG cultivars inoculated with ergot isolates from PRG exhibited significantly greater disease incidence and a greater number of sclerotia than the same cultivars inoculated with ergot isolates from KBG (Table 4). These results indicate potential differences in the ability of ergot populations from these two hosts to infect PRG.
- **Objective 4.** Disease incidence based on honeydew appearance (Fig. 5) indicated that micro-plots that were located closer to the point source of inoculum contained more infected plants. However, due to the small sample size a significant association could not be established. A second year study has been planned with increased sample size to examine the importance of secondary spread via honeydew.

  The first-year survey of commercial grass seed fields indicated that Dipteran insects were the most abundant group (Fig. 6). A significant positive association existed between insect abundance and ergot incidence in PRG fields (Fig. 7). Three families of flies (Calliphoridae, Dolichopodidae and Muscidae) were prominent. Microscopic examinations revealed the presence of ergot conidia inside insect guts which was then confirmed with high-fidelity PCR (Fig. 8). High-fidelity PCR detected *Claviceps* in flies (35% of 57 tested) and moths (27% of 36 tested).

Impacts:

This project has focused on developing novel components of an integrated disease management program to reduce ergot disease, including: chemical control, disease monitoring and forecasting, host plant resistance, and insect vector management. Information from the initial fungicide trial has identified products and application methods that can be useful for ergot management. Continued studies on fungicide efficacy will provide additional information on appropriate application strategies and confirm the efficacy of specific fungicides to manage this disease. Likewise, the development of a disease prediction model will be a useful tool for decision making regarding protective fungicide applications during anthesis and may help reduce the number of fungicide applications required. Some cultivars were identified that appeared to escape periods of high ascospore production. Further tests on these cultivars will help in understanding the mechanisms of host plant resistance and provide information that can aid

breeders in the development of new cultivars that exhibit reduced ergot infection. Our results suggest that insect vectors are important factors in the secondary spread of this disease. Future studies on insect management may provide information on reducing disease spread via honeydew.

We presented our findings at the Grass Seed Field Day held at HAREC in May 2014 and ergot disease symptoms and identification was presented to a group of master gardeners at HAREC in June 2014. Oral and poster presentations were made at the American Phytopathological Society Pacific Division meeting in Bozeman, MT (July 2014) and at the Entomological Society of America meeting in Portland, OR (November 2014), respectively. In the near future, extension publications and presentations will be made available to growers in the Pacific Northwest region.

Relation to Other Research:

This project involves the commitment of a diversity of disciplines focused on developing a better understanding of this pathogen. The ergot research team consists of plant pathologists, molecular biologists, agronomists, and entomologists. Over the past few years we have made significant progress in our understanding of ergot, and we hope to gain an even greater understanding of ergot biology and epidemiology in the upcoming years.

Acknowledgements:

We would like to thank Washington Turfgrass Seed Commission and Oregon Seed Council for funding. We are also thankful to Columbia Basin Grass Seed Growers and Union County Grass Seed Growers for their continued support and participation for the success of this project. The technical support provided by Ronda Simmons and Robert Cating, as well as the assistance from staff members at HAREC, is greatly appreciated.

Table 1. Predictive accuracy of environmental variables used to forecast the occurrence of at least one, ten, or 100 ergot ascospores

	Spores present/absent			≥10 Spores present/absent			≥100 Spores present/absent		
Environmental variable	**Correct predictions**	**False positives**	**False negatives**	**Correct predictions**	**False positives**	**False negatives**	**Correct predictions**	**False positives**	**False negatives**
Minimum air T = 41 to 54°F	60.3%[1]	16.9%	22.8%	64.0%	21.2%	14.8%	60.3%	29.1%	10.6%
Maximum soil T = 59 to 72°F	74.1%	0.0%	25.9%	86.2%	0.0%	13.8%	88.9%	4.8%	6.3%
Minimum soil T = 57 to 70°F	82.5%	3.7%	13.8%	84.1%	9.0%	6.9%	81.5%	16.4%	2.1%
Mean soil T = 57 to 70°F	77.8%	0.5%	21.7%	90.0%	0.5%	9.5%	88.4%	7.4%	4.2%
Daily soil degree days = 11 to 20	74.6%	0.0%	25.4%	86.8%	0.0%	13.2%	89.4%	4.8%	5.8%
Mean dew point = 37 to 50°F	67.7%	15.4%	16.9%	69.3%	20.6%	10.1%	66.7%	28.0%	5.3%

[1] Chi-square result was not significant at $P < 0.05$. All other chi-square values (not shown) were significant at $P < 0.05$.

Table 2. Predictive accuracy of an environmental favorability index (EFI) model used to forecast the occurrence of at least one, ten, or 100 ergot ascospores[1]

	Spores present/absent			≥10 Spores present/absent			≥100 Spores present/absent		
Environmental Favorability Index (EFI)	**Correct predictions**	**False positives**	**False negatives**	**Correct predictions**	**False positives**	**False negatives**	**Correct predictions**	**False positives**	**False negatives**
EFI ≥ 1	78.9%	15.3%	5.8%	77.3%	22.2%	0.5%	66.1%	33.9%	0.0%
EFI ≥ 2	81.5%	3.7%	14.8%	86.2%	7.4%	6.4%	83.6%	14.8%	1.6%
EFI ≥ 3	73.5%	0.0%	26.5%	85.7%	0.0%	14.3%	90.5%	3.7%	5.8%
EFI ≥ 4	65.1%	0.0%	34.9%	77.2%	0.0%	22.8%	86.2%	1.6%	12.2%

[1] All chi-square values (not shown) were significant at $P < 0.0001$.

Table. 3. Variety, source, and anthesis timing of the nine perennial ryegrass varieties evaluated for ergot susceptibility[1]

Cultivar	Anthesis begin date (Julian day)	Anthesis end date (Julian day)	Anthesis duration (days)
Applaud II	135 e	165.2 bcd	30.2 ab
1G2	137.7 d	168.2 bc	30.5 ab
Integra II	132.5 f	167.2 bc	34.7 a
PST-2M20	139.2 c	175.2 a	35.5 a
Silver Dollar	139.2 cd	164.5 cd	25.2 bcd
Quickstart II	128.5 g	163 d	34.5 a
Top Hat 2	142.5 b	165 bcd	22.5 d
Derby Extreme	145.2 a	174.7 a	29.5 abc
Esquire	142.7 b	168.7 b	26 bcd
Fiesta 4	144.5 a	167.5 bc	23 cd
SR 4600	145.5a	165.7 bcd	20.2 d
Karma	146 a	168.7 b	22.7 cd
F-value (df)	315.7 (11)	21.72 (11)	15.1 (11)
P- value	<0.0001	<0.0001	<0.0001

[1] Means followed by the same letters are not statistically different using Tukey's comparision.

Table 4. Incidence of ergot infection and the mean number of sclerotia after inoculating 12 perennial ryegrass (PRG) cultivars with a mix of ergot isolates from PRG and Kentucky bluegrass (KBG)

	PRG ergot		KBG ergot	
PRG cultivar	Incidence (%)	Mean sclerotia	Incidence (%)	Mean sclerotia
Applaud II	17.6	23.3	4.2	3.3
1G2	21.3	41.0	3.3	1.3
Integra II	26.6	54.3	7.0	4.0
PST-2M20	18.7	20.7	0.5	H[1]
Silver Dollar	22.0	58.0	8.3	10.3
Quickstart II	18.5	27.0	2.9	1.7
Top Hat 2	18.4	26.0	2.2	1.7
Derby Extreme	18.1	42.0	1.3	0.7
Esquire	25.5	34.3	12.1	18.7
Fiesta 4	24.0	34.7	0.4	0.3
SR 4600	24.5	60.6	6.7	6.0
Karma	20.1	31.7	2.7	1.0

[1] H = only honeydew observed.

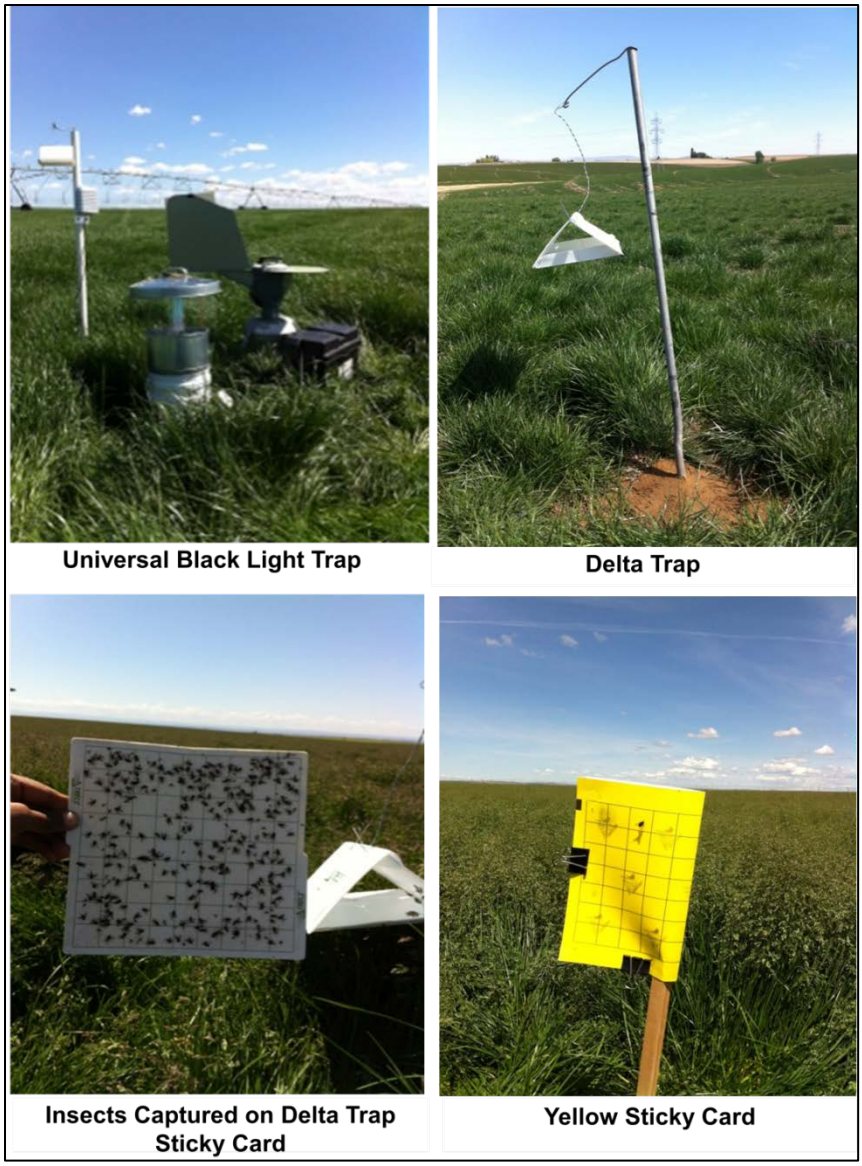

Fig. 1. Insect traps used to monitor insect abundance in grass seed fields.

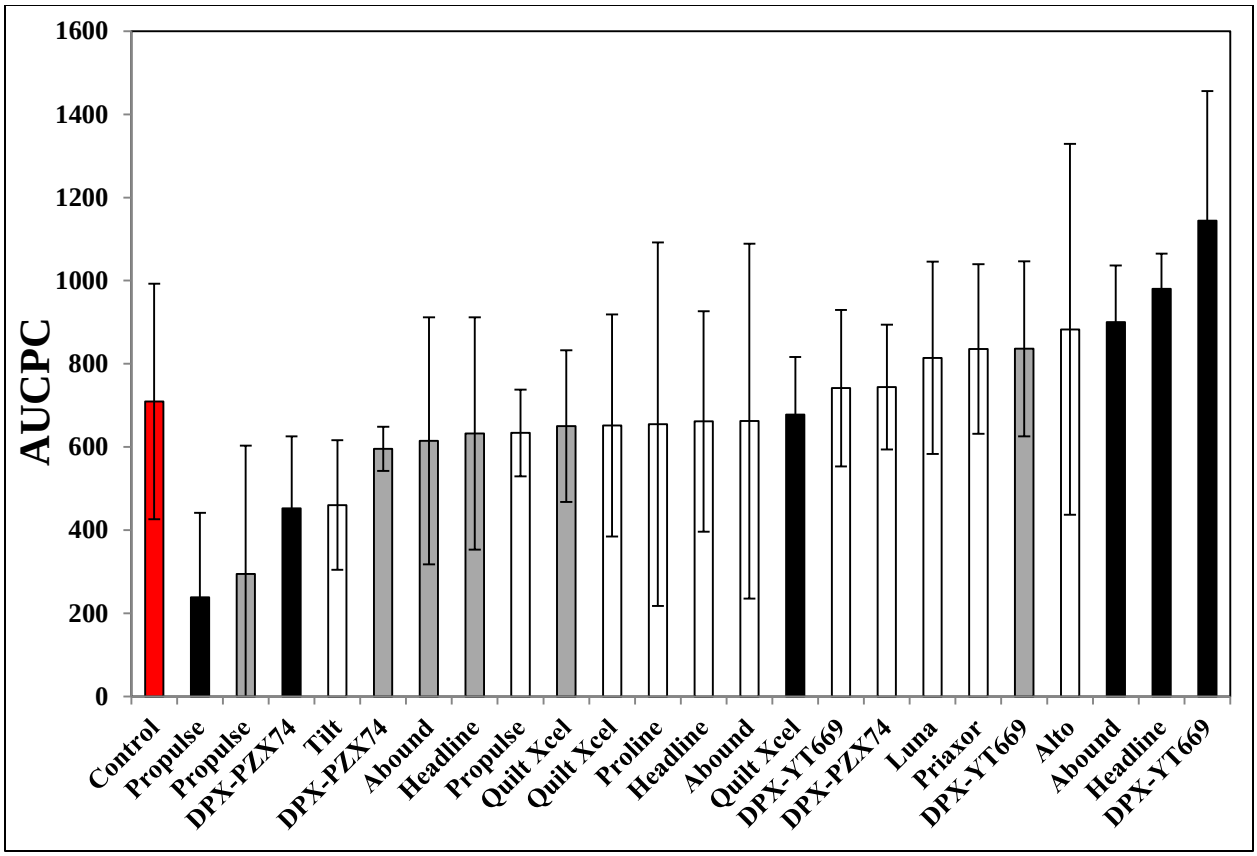

Fig. 2. Mean area under capitula production curve (AUCPC) values in experimental plots infested with ergot sclerotia from perennial ryegrass in October 2013 and treated with soil-applied fungicides in fall 2013 (grey bars), spring 2014 (white bars), or in both fall 2013 and spring 2014 (black bars). The red bar represents the water-treated control.

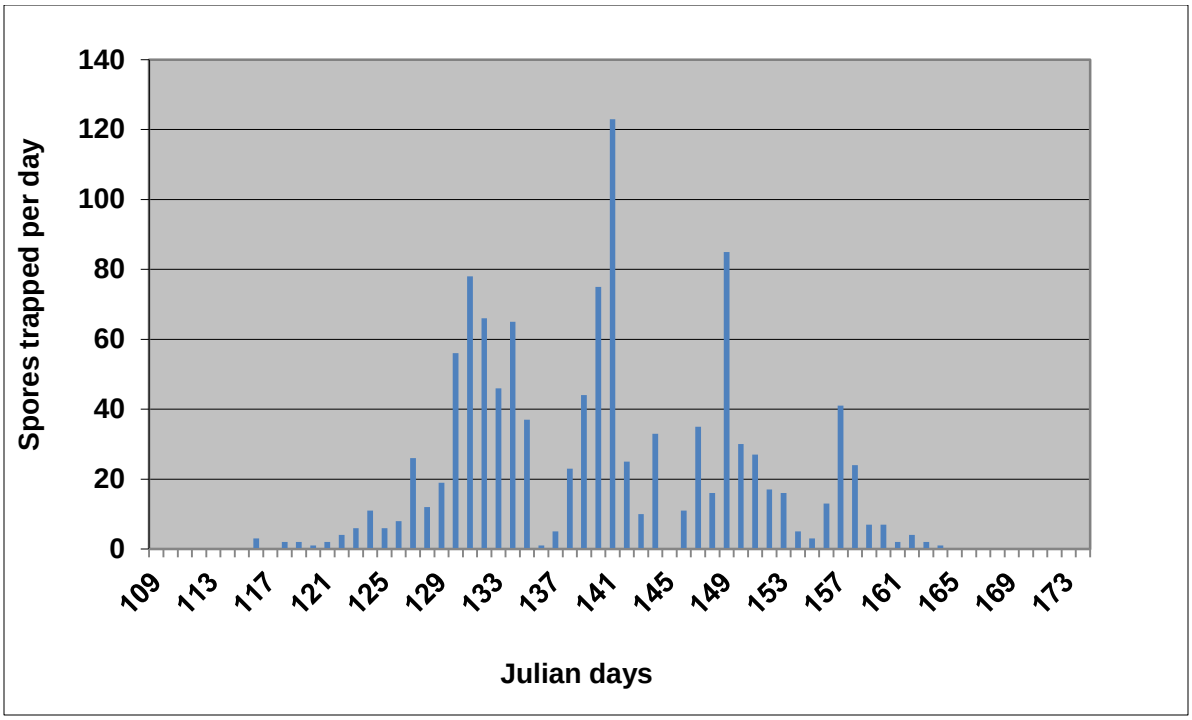

Fig. 3. Timing of ascospore release in the perennial ryegrass variety trial plots located at the Hermiston Agricultural Research and Extension Center.

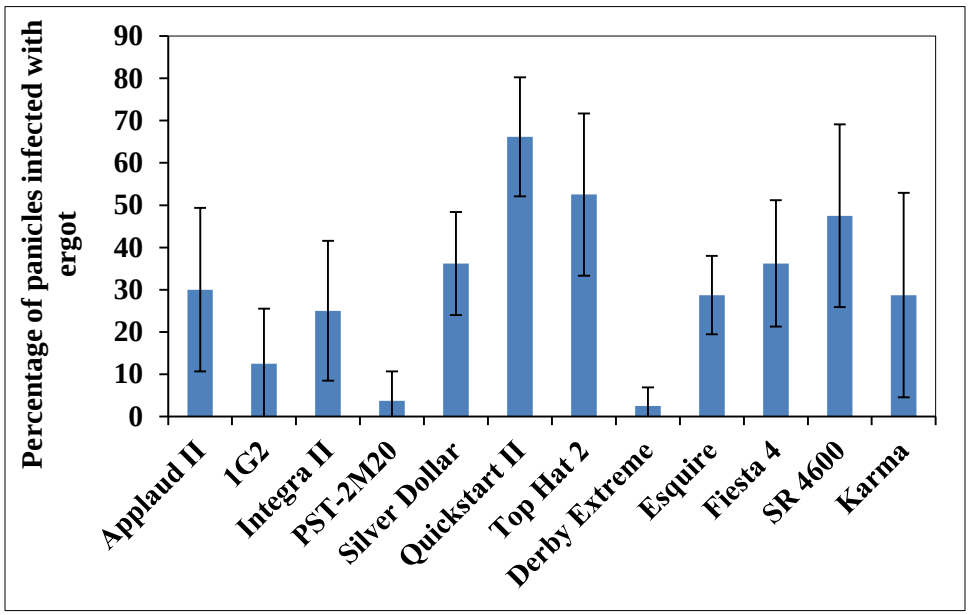

Fig. 4. Ergot incidence in 12 perennial ryegrass (PRG) varieties based on the percentage of flowers exhibiting honeydew symptoms.

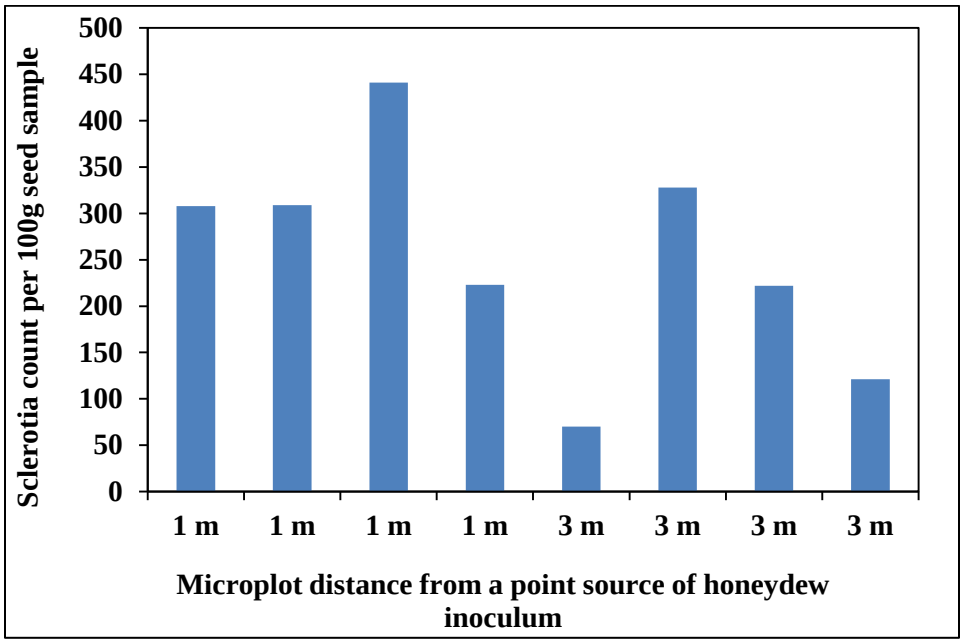

Fig. 5. Ergot severity in perennial ryegrass micro-plots located 1m (3.2 ft) and 3m (9.8 ft) away from a point source of honeydew inoculum (chi-square = 2.95, df= 7, *P* = 0.88).

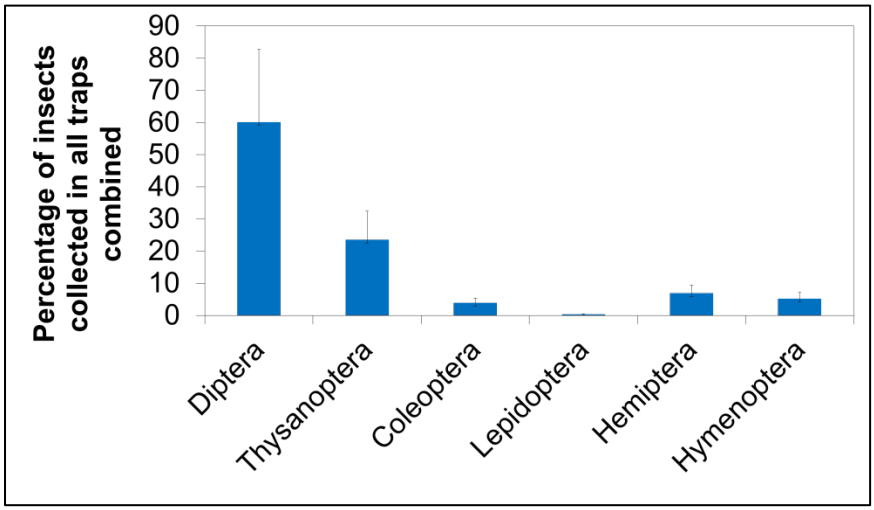

Fig. 6. Relative abundance of insect groups collected from commercial Kentucky bluegrass and perennial ryegrass seed fields during May and June 2014.

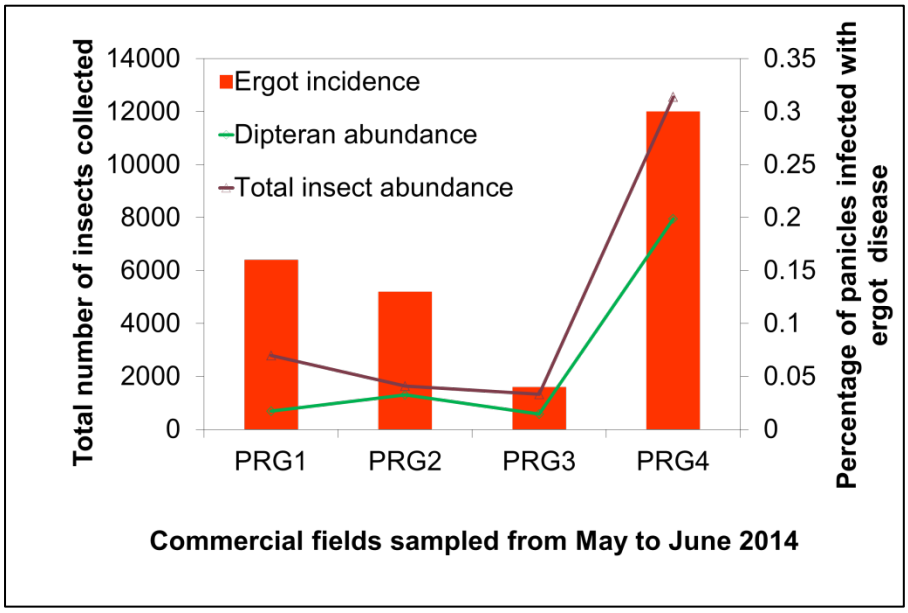

Fig. 7. A significant positive correlation (r = 0.9, P< 0.05) existed between insect abundance and ergot incidence in four commercial perennial ryegrass (PRG) fields during May and June 2014. However no association could be established in Kentucky bluegrass fields because ergot incidence was negligible (data not shown).

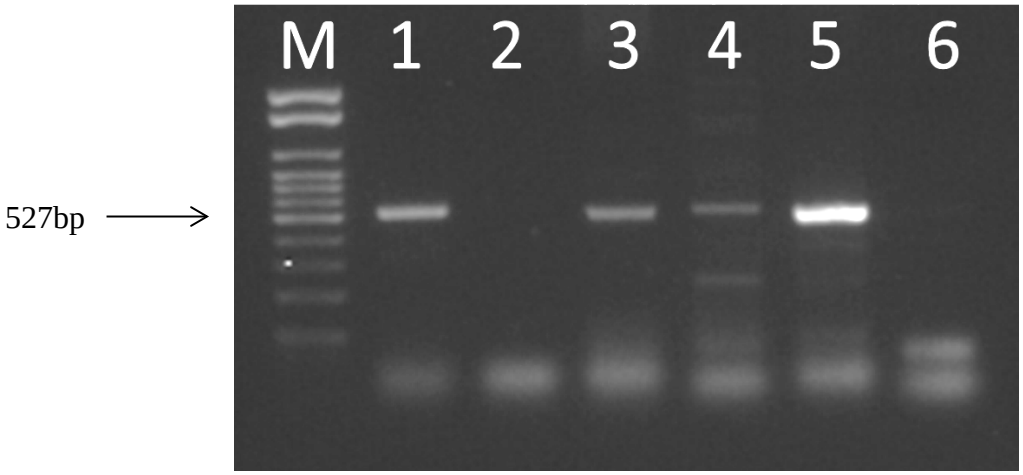

Fig. 8. Agarose gel results obtained from a high-fidelity polymerase chain reaction used to detect the presence of ergot on/in insects. M= molecular marker; lane 1 = positive control; lane 2 = negative control; Lanes 3-6 = insect samples. The ergot beta-tubulin gene amplification product occurs at ~527bp length.