Report of Accomplishments from Research Funded by the Washington Turfgrass Seed Commission (2012-2015):

Title: Controlling Ergot in Kentucky Bluegrass

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Introduction:

Ergot, caused by the fungal pathogen *Claviceps purpurea*, is a floral disease of grasses and a persistent problem in many Kentucky bluegrass (KBG) and perennial ryegrass (PRG) seed production systems (Alderman et al. 1996, Alderman et al. 1998). The fungus has a very wide host range in North America, including important grains grown for human and livestock consumption, as well as forage, turf, and weedy grasses (Alderman et al. 2004). The pathogen infects the unfertilized flowers of grasses and grains and transforms seed into fungal structures called sclerotia. Sclerotia overwinter and germinate to produce fruiting bodies called capitula which in turn release hundreds to thousands of airborne ascospores that serve as primary inoculum during the following season. In addition to ascospores, asexual spores called conidia are produced in large numbers and mix with plant sap exuded from infected ovaries to form a substance referred to as honeydew. Honeydew can serve as secondary inoculum if splash- or insect-dispersed to uninfected flowers within a season. In addition to yield losses due to seed replacement, the repeated cleaning required to remove ergot sclerotia from seed lots results in increased seed loss during cleaning operations as well as additional time and labor costs. Highly infested seed lots are rejected for certification and seed screenings (plant material left over from the seed cleaning process) cannot be pelletized to be sold as feed due to high amounts of ergot alkaloids, which are toxic to humans and livestock. One of the major challenges in ergot control is the extremely large number of sclerotia that can be left in perennial grass seed fields after harvest; one study found between 16,000 and 480,000 sclerotia/acre that were deposited in perennial ryegrass fields after harvest (Dung et al. 2015).

Ergot control in grass seed production in WA and OR has been improved in recent years, but multiple protective fungicide applications are required during flowering. Growers often make multiple fungicide applications in an effort to prevent and control the disease, reportedly spending $60 to 75/ acre. Only two fungicide chemistries are labeled for ergot control in grass grown for seed in the Pacific Northwest:

azoxystrobin (FRAC 11) and propiconazole (FRAC 3). Taking into account the repeated applications of similar fungicides for powdery mildew and rust control in grass seed crops, the potential for resistance development is increased in fungal pathogens affecting cool-season grasses.

Over the last decade a collaborative research effort at Oregon State University and USDA-ARS has contributed to the foundation for the development of a multi-tactic integrated pest management (IPM) approach for ergot (Fig.1) that incorporates cultural control, host plant resistance, disease prediction modelling, and chemical control to minimize the impact of ergot in the grass seed industry (Alderman 2015). Cultural control measures for ergot include planting ergot-free seed, crop rotation, seed cleaning, and controlling weedy hosts.

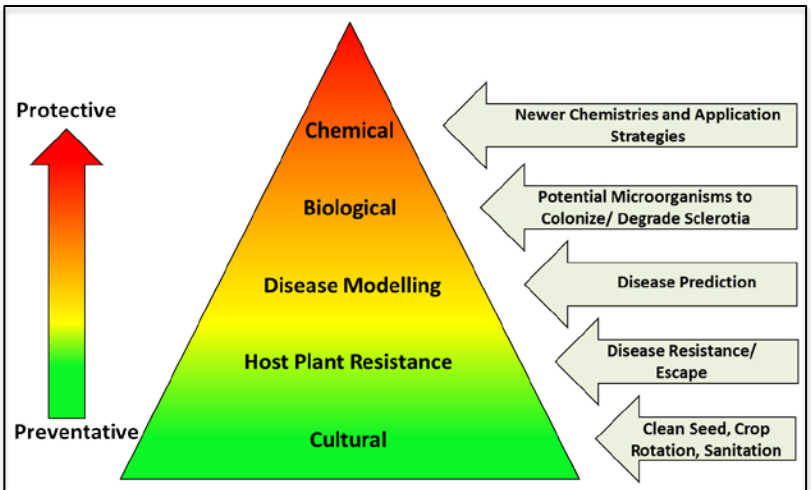

Fig. 1. Integrated pest management pyramid summarizing our research approaches to improve ergot management in cool-season grass seed crops.

Cultivars with shortened and uniform flowering periods, or cultivars that flower outside of peak periods of ascospore production, have the potential to escape ergot infection and may form a basis for breeding cultivars that are less susceptible to ergot (Kaur et al. 2015). Disease models to predict ascospore release, more sensitive methods of detecting ascospores, and frequent and effective outreach to growers could all be used in conjunction to improve the timing and reduce the number of protective fungicide applications (Dung et al. 2014; Dung 2015). New fungicide chemistries and novel application strategies have also been explored (Dung et al. 2012). Our current research objectives are listed below.

OBJECTIVES:

- **Objective 1.** Determine the efficacy of soil-applied fungicides to reduce sclerotia germination and continue to evaluate new chemistries to protect flowers from ascospores during anthesis.
- **Objective 2.** Develop a predictive model based on crop phenology and environmental factors which favor ergot development to provide growers with a decision aid for ergot management.
- **Objective 3.** Evaluate KBG and PRG cultivars for the potential to escape or resist ergot infection.
- **Objective 4.** Understand the significance of honeydew and the role of insects in ergot dispersal.

PROCEDURES:

Objective 1) Evaluation of fungicides to manage ergot. This study was conducted under field and laboratory conditions and included three sub-objectives:

1.1. Use of soil applied fungicides to reduce sclerotia germination. Plots of KBG cultivar 'Midnight', with four replicates per treatment, were established at HAREC in September 2014. The field was divided into plots 3.3 ft. long and spaced 3.3 ft. apart, with 7 rows per plot. Each plot was infested in October 2014 with 100 sclerotia collected from PRG. Treatments consisting of fall, spring, and fall+spring applications of 12 fungicides (Table 1) and a non-treated control were applied with a CO_2 backpack sprayer at labeled rates in a volume of 400 gal/acre. Fall treatments were applied in October 2014. Spring treatments were applied in April 2015 and the number of ergot fruiting bodies (capitula) was counted

from May to June 2015. 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) and multiple comparisons were made using Tukey's test.

1.2. Evaluation of alternative fungicides to protect flowers from ergot infection during anthesis. Plots of PRG cultivar 'Derby Extreme' were established at HAREC in September 2014. Four replicated plots (20 x 3.5 ft.) with 10 ft. buffer zones were arranged in a randomized complete block design. Treatments were applied during anthesis at Feekes stage 10.51 (first appearance of stigmas/anthers). Fungicide treatments included Propulse (fluopyram + prothioconazole; Bayer CS), Priaxor (pyraclostrobin + fluxapyroxad; BASF), Solatenol (benzovindiflupyr; Syngenta), Fontelis (penthiopyrad; DuPont), an industry standard consisting of QuiltXcel (azoxystrobin + propiconazole; Syngenta), and a non-treated water control. Three applications were made at weekly intervals using a CO_2 backpack sprayer at labeled rates in a volume of 60 gal/acre. When honeydew appeared, the number of seed heads showing symptoms of infection was determined out of 40 heads collected randomly from each plot. At harvest, 40 seed heads were randomly collected from each plot and disease incidence and disease severity were calculated based on the number of seed heads containing ergot sclerotia and the number of sclerotia present in each seed head, respectively. Data were analyzed using ANOVA and multiple comparisons were made using Tukey's test.

1.3. Petri dish assays. Three laboratory assays were conducted to determine: a) the effect of pre-irrigation on soil-applied fungicide efficacy; b) the effect of fungicides on germinated sclerotia and capitula; and c) the efficacy of new fungicide chemistries on sclerotia germination.

a) Impact of soil moisture on the efficacy of soil-applied fungicides. A total of 3 fungicides were selected for this study based on their efficacy in a previous trial. Treatments consisted of azoxystrobin at 15.5 oz/acre (highly effective in previous tests), pyraclostrobin at 12 oz/acre (moderately effective in previous tests), picoxystrobin+cyproconazole at 13.7 oz/acre (slightly effective in previous tests), and a non-treated water control. Soil moisture treatments consisted of dry (no water applied prior to fungicide treatments) and wet (water applied prior to fungicide treatments). Twenty sclerotia from PRG were preconditioned on moist sterile soil in petri plates at 41°F for six weeks. The plates were allowed to dry for 6 days and fungicides were applied to one half of the plates (dry treatment). The remaining plates were moistened with sterile water immediately before fungicide applications (wet treatment). After 3 days, plates were moistened with sterile water and incubated at 60°F for 3 weeks. The number of germinating sclerotia and capitula were counted weekly for 6 weeks and data were converted to Area Under Sclerotia Germination Curves (AUSGC) and AUCPC, respectively. Each treatment was replicated three times using a petri plate containing 20 sclerotia. The experiment was arranged as a randomized complete block design and data were analyzed as a two-way factorial using ANOVA with fungicide and soil moisture as treatment factors. Multiple comparisons were performed using Tukey's test.

b) *Effect of fungicides on germinated sclerotia and capitula*. PRG sclerotia were placed on 25 g of sterilized soil contained in a petri dish. Sclerotia were preconditioned on moist sterile soil at 41°F for six weeks followed by incubation at 60°F for seven weeks. Petri plates containing at least 10 germinated sclerotia were used for this test. Fungicide treatments were made when capitula were < 1 week old. Four replicate plates were used for each treatment. The six fungicide treatments included: Mettle (tetraconazole; Gowan) at 10 oz/acre , Torino (cyflufenamid; Gowan) at 3.4 oz/acre, Zing! (zoxamide+chlorothalonil; Gowan) at 30 oz/acre, Solatenol (benzovindiflupyr; Syngenta) at 0.4 oz a.i./acre, Fontelis (penthiopyrad; DuPont) at 24 oz/acre, QuiltXcel (azoxystrobin + propiconazole; Syngenta) at 14 oz/acre, and a non-treated water control. The data on number of capitula that survived after fungicide treatment were collected for three weeks.

c) *Effect of new chemicals on sclerotia germination*. Sclerotia were preconditioned in moist sterile soil at 41°F for six weeks during August 2015. Fungicide treatments were then applied and the sclerotia were incubated at 60°F for seven weeks. The number of germinating sclerotia and capitula are being recorded and data will be analyzed using ANOVA. Comparisons to the non-treated control will be performed using Dunnett's test.

Objective 2) Identifying environmental factors which favor ergot spore production and development of a predictive model

This year seven Burkard spore traps were deployed in three grass seed production areas: the Columbia Basin (OR and WA), the Grande Ronde Valley (OR), and central Oregon (Table 2). These spore traps captured airborne spores (and other material) continuously. Personnel collected the spore trap samples and performed trap maintenance on a weekly basis. 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 AgriMet and AgWeatherNet 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) Evaluation of KBG and PRG cultivars for the potential to escape or resist ergot infection. This work was performed under field and greenhouse conditions. It was hypothesized that cultivars which flower before or after peak ergot spore production, or those with shortened periods of anthesis, would escape infection more than those which flower when ergot spores are present at high levels.

3.1. Evaluation of KBG cultivars for disease escape potential. KBG plots (26 ft. long and consisting of 6 rows of plants) were established at COARC in August 2014. A total of 12 cultivars ('Blue Ghost', 'Gateway', 'Shamrock', 'Bluechip', 'Gladstone', 'Nuglade', 'PST-K4-7', 'Fielder', 'Midnight II', 'Jumpstart', 'Right', and 'DB-1013') were replicated four times each and arranged in a randomized complete block design. The border of the plot area was artificially infested in October 2014 with KBG sclerotia collected from seed lots produced in central Oregon. A Burkard spore trap was placed in the plot area to capture spores between April 10, 2015 and July 1, 2015. Weather data were collected from the AgriMet MRSO weather station located at COARC. The timing and duration of anthesis was determined as described above. Disease incidence (number of infected seed heads) and severity (number of sclerotia) were determined from a random sample of 100 seed heads collected from each plot at harvest. Data were analyzed as described above. A study site was also planted in the Grande Ronde Valley in April 2015 to further evaluate disease escape potential of 8 KBG cultivars including: Abbey, Endurance, Baron, Midnight II, Jumpstart, Prosperity, Thermal Blue and Wildhorse. Evaluations in the Grande Ronde Valley will begin in 2016.

3.2. Evaluation of PRG cultivars for disease escape potential. A field trial was conducted at HAREC to determine the potential for PRG cultivars to escape ergot infection. Research plots consisting of 12 PRG cultivars ('Applaud II', 'IG2', 'Integra II', 'PST-2M20', 'Silver Dollar', 'Quickstart II', 'Top Hat 2', 'Derby Extreme', 'Esquire', 'Fiesta 4', 'SR 4600', 'Karma') were established in September 2014. Each plot was 30 ft. long and consisted of 7 rows of plants. Plots were replicated four times and arranged as a randomized complete block design. Each plot was infested in October 2014 with 200 sclerotia collected from PRG seed lots harvested in 2014. A Burkard spore trap was placed near the plots to determine the timing of ascospore release between April 3, 2015 and June 15, 2015. Weather data were recorded from the AgriMet HRMO weather station located at HAREC. The timing and duration of anthesis for each cultivar were recorded to determine disease escape potential. At the appearance of honeydew symptoms, 40 seed heads were randomly collected from each plot and the number of seed heads showing symptoms of infection was determined. At harvest, disease incidence and disease severity

were calculated. 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. Data were analyzed using ANOVA and multiple comparisons were made using Tukey's test.

3.3. Evaluation of ergot resistance in KBG and PRG. Greenhouse experiments were initiated to evaluate ergot resistance in a) KBG and b) PRG cultivars to ergot isolates from both hosts. The KBG and PRG trials were conducted at COARC and HAREC, respectively.

a) Evaluation of ergot resistance in KBG cultivars. Seeds of 12 KBG cultivars (listed above) were planted in August, 2014 into 4 in. pots containing peat-based potting mix. Plants were maintained in an unheated greenhouse at COARC to vernalize during the winter. Inoculum, consisting of ergot conidia from isolates collected from KBG and PRG, were produced in liquid culture and stored in 60% sucrose at -80°C until needed for artificial inoculations.

b) Evaluation of ergot resistance in PRG cultivars. Seeds of 12 PRG cultivars (listed above) were planted in August, 2014 into 4 in. pots containing peat-based potting mix. Plants were maintained in an unheated greenhouse at HAREC to vernalize during the winter. Inoculum, consisting of ergot conidia from isolates collected from KBG and PRG, were produced in liquid culture and stored in 60% sucrose at -80°C until needed for artificial inoculations.

Objective 4) Understanding the significance of honeydew and the role of insects in ergot dispersal. This study was categorized under two sub objectives:

4.1. Spatial spread of ergot from a point source of honeydew inoculum. An isolated field plot of PRG was planted at HAREC in September 2014. Four sub plots representing four replicates (15 x 9 ft.) were established in the spring 2015 and each subplot was further divided into six micro plots (3 ft. x 3 ft.) with 3 ft. buffer zones. An infected plant with honeydew was placed in the center of the field plot so that six micro plots were located at the distance of 3 ft., 6 ft., 9 ft., 12 ft., 15 ft. and 18 ft. apart from the infected plant. Ergot infection and spread in the micro plots was monitored during and after anthesis to determine the rate of ergot spread from a single source of honeydew. Data was analyzed using chi-square goodness of fit analyses.

4.2. Role of insects in ergot dispersal. Insect traps (universal black light traps, delta traps, yellow sticky cards, and sweep nets) were deployed to monitor insect densities at 2 KBG fields and 3 PRG fields. PRG fields consisted of one commercial field located in Benton Co., WA, a commercial field located in Umatilla Co., OR, and the PRG cultivar trial located at HAREC. The two commercial KBG fields were both located Benton Co., WA. Sampling was carried out at weekly intervals from April to June 2015. Insects were sorted, counted and stored at −20ºC until microscopic examination for the presence of fungal spores. 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. The association between insect densities and ergot incidence was calculated by correlation analyses. Molecular techniques based on polymerase chain reaction (PCR) were used to confirm the presence of fungal spores carried by insects.

A preliminary insect transmission study was also conducted to determine the potential for insect-mediated transmission of ergot from infected to healthy plants. A clean fly colony was established and10 freshly emerged adults were allowed to feed on a sterilized cotton swab soaked with a sugar solution containing 10^6 conidia/ml. The flies were exposed to the conidia solution for 12-, 24-, and 48 h and then caged with inflorescences of healthy PRG 'Quickstart' plants at 10.51 Feekes stage. Flies were caged with the

inflorescences in a greenhouse for up to 1 wk. Due to a limited number of flowering plants in the greenhouse, only one replicate per treatment was tested.

RESULTS AND ACCOMPLISHMENTS:

Objective 1) Evaluation of fungicides to manage ergot.

1.1. Use of soil applied fungicides to reduce sclerotia germination. Significant reductions in sclerotia germination were not observed compared to the water-treated control plots (Fig. 2). Despite the lack of statistical differences, fall applications of Propulse reduced AUCPC values by 75% and spring and fall+spring applications resulted in 40% and 48% reductions, respectively. These results are consistent with results obtained in 2013 and 2014, where Propulse applications reduced AUCPC values by 55 and 76%, respectively. Similarly, fall+spring application of Abound reduced AUCPC values by 76%.

1.2. Evaluation of alternative fungicides to protect flowers from ergot infection during anthesis. Applications of Propulse, Quilt Xcel, and Priaxor during anthesis significantly reduced ergot honeydew (Fig. 3) ($P = 0.002$). Disease severity was significantly reduced in all fungicide treatments compared to the non-treated water control ($P = 0.0079$).

1.3. Petri dish assays.

a) *Impact of soil moisture on the efficacy of soil-applied fungicides.* A significant effect of fungicide ($P < 0.0001$) was observed on sclerotia germination (AUSGC) and capitula production (AUCPC) (Table 3). A significant effect of soil moisture was not observed for AUSGC ($P = 0.13$) or AUCPC ($P = 0.37$) and significant fungicide x soil moisture interactions were not observed for either AUSGC ($P = 0.09$) or AUCPC ($P = 0.23$). However, AUSGC and AUCPC values were lower in azoxystrobin and pyraclostrobin treatments that were applied when the sclerotia were dry, though the differences were not significant.

b) *Effect of fungicides on germinated sclerotia and fruiting bodies*. Significant effects of post-germination fungicide applications were not observed (Table 4). The tested fungicides did not kill or significantly reduce the viability of ergot fruiting bodies.

c) *Effect of new chemicals on sclerotia germination*. This assay is currently in-progress. Plates were transferred to 16°C by the end of September. Data collection is still in progress.

Objective 2. Identifying environmental factors which favor ergot spore production and development of a predictive model

Kentucky bluegrass – Columbia Basin and Grande Ronde Valley. Only 7 spores were captured at each of the two field locations in the Columbia Basin of Washington. This is the fourth year in a row in which relatively few (between 7 and 50) ascospores have been captured in commercial fields, preventing the development of a predictive model. This leads to the question: how important are airborne ascospores to ergot epidemics in KBG in the Columbia Basin of Washington? In the Grande Ronde Valley of Oregon, only 53 spores were captured at one location and 194 spores were captured at the second location. Model development using data collected in the Grande Ronde Valley between 2012 and 2015 is currently in progress.

Kentucky bluegrass – Central Oregon. Data for several environmental variables collected from the Watchdog data logger placed in the field and the MRSO weather station at COARC were significantly ($P \leq 0.05$) and positively correlated with ergot spore production (Table 5). Logistic regression using data from the field data logger identified a model that included minimum air temperature, minimum and maximum soil temperatures, dew point, and soil moisture that predicted the presence of ergot spores with over 92% accuracy. Using data from the MRSO weather station, logistic regression identified a model

that included minimum and mean air temperature and minimum, maximum and mean soil temperatures that predicted the presence of ergot spores with over 91% accuracy. Local regression and box-and-whisker plots were used to identify upper and lower threshold values for environmental factors significantly correlated with spore production. A minimum air temperature greater than 41° F, minimum soil temperature greater than 50° F, maximum soil temperature less than 71° F, dew point between 40 and 50° F, and a volumetric water content between 18 and 25% in the field were associated with ergot spore production. These results are consistent with data collected in PRG fields of the Columbia Basin and data obtained from controlled studies conducted in incubation chambers. Based on the results obtained in this study and in other trials, minimum air temperature, minimum and maximum soil temperatures, dew point, and soil moisture appear to be the important factors contributing to ergot spore production. Several years of data will be required to develop and test a final model for ergot spore prediction in Central Oregon Kentucky bluegrass seed production.

Perennial ryegrass – Columbia Basin. Significant correlations ($P < 0.05$) were observed between spore counts and minimum ($r = 0.18$), maximum ($r = 0.18$), and mean ($r = 0.18$) soil temperatures were observed in 2015, which is consistent with results obtained in 2013 and 2014 (Table 6). The cumulative environmental favorability index (EFI) model that was developed in 2014 was tested against data collected in 2015. The EFI model includes mean soil temperature between 63 and 73° F, maximum soil temperature between 66 and 81° F, minimum soil temperature between 55 and 70° F, daily soil degree days between 8 and 14, and mean dew point between 37 and 48° F. A cumulative EFI value of 2 correctly predicted the occurrence of at least one spore with an accuracy of 75% in 2015 and with an accuracy of over 80% when data were combined from 2013, 2014, and 2015 (Table 6). These results suggest that predictive models can be a useful tool to predict ergot ascospore production in the Columbia Basin.

Objective 3) Evaluation of PRG and KBG cultivars for the potential to escape or resist ergot infection.

3.1 Evaluation of KBG cultivars for disease escape potential. Significant differences in anthesis initiation date, anthesis termination date, and anthesis duration were observed among the 12 KBG cultivars evaluated in central OR ($P \leq 0.003$) (Table 7). Among the cultivars tested, Midnight II exhibited the highest ergot incidence and severity, while Jumpstart and Fielder exhibited the lowest ergot incidence and severity (Table 7). A significant positive correlation was observed between anthesis initiation date and ergot incidence ($P = 0.002$; $r = 0.44$) and severity ($P = 0.01$; $r = 0.37$) was observed, suggesting that cultivars which initiated flowering earlier in the season exhibited reduced ergot in central Oregon.

3.2. Evaluation of PRG cultivars for disease escape potential. One replicate each of 'Silver Dollar', 'SR4600' 'Karma', 'Integra II', 'PST-2M20', 'Top Hat2', 'Derby Extreme' and two replicates of 'Esquire' were damaged by an accidental leak of Vapam from a neighboring experimental field. Despite the loss of some plots, there was a significant difference between the time of flowering (e.g. anthesis initiation, 50% anthesis, anthesis termination) among the cultivars evaluated (Table 8). Consistent with our previous findings in 2014, PRG cultivar 'Quickstart II' initiated flowering significantly earlier in the season compared to the other cultivars (Table 7). The anthesis initiation date of 'Quickstart II' (May 4, 2015) coincided with the first peak of ascospore release that occurred between May 2 and May 4, 2015 and this cultivar exhibited the highest disease incidence among the cultivars evaluated. In contrast, 'PST-2M20' and 'Derby Extreme' exhibited significantly less disease incidence ($P < 0.001$) based on the incidence of honeydew (Table 8), which was also consistent with the results obtained in 2014. A significant negative correlation was observed between ergot infection and anthesis initiation date ($P < 0.001$; $r = -0.54$) suggesting that ergot was reduced in cultivars that initiated anthesis later in the year; this result is also similar to observations made in 2014.

3.3. Evaluation of ergot resistance in KBG and PRG. None of the KBG or PRG cultivars flowered in the greenhouses at COARC and HAREC, respectively, so inoculations could not be performed this year.

Next year KBG and PRG plants will be transplanted from newly established field plots into the greenhouse in March 2016, prior to the appearance of ergot spores, to ensure vernalization requirements are met for flowering.

Objective 4) Understanding the significance of honeydew and the role of insects in ergot dispersal.
4.1. Spatial spread of ergot from a point source of honeydew inoculum. Disease incidence based on honeydew appearance (Table 9) was not significantly different among the micro-plots spatially separated approximately 20 ft. away from the point source of inoculum suggesting honeydew may be dispersed at least 20 ft. via water or insect movement.

4.2. Role of insects in ergot dispersal. The second-year survey of commercial grass seed fields indicated flies were the most abundant group (47% of total captured), followed by thrips (41% of total captured) (Fig. 4). A significant positive correlation ($r = 0.4$, $P < 0.05$) (Fig. 5) existed between insect abundance and ergot severity in commercial grass seed fields during April to June 2015. High-fidelity PCR detected ergot spores in flies (10 of 44 tested) and moths (4 of 21 tested). Work is currently in progress to determine the relationships between different *Claviceps* spp. that were found on different hosts and locations. No infection was detected in the preliminary insect transmission study. Factors such as the effect of ergot alkaloids on insect behavior and biology, host plant phenology and fertility status, or environmental conditions in greenhouse may have impacted the transmission process. Additional replicates will be tested in the future once grass plants start flowering under greenhouse settings.

CONCLUSIONS AND IMPACTS
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. 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. Several fungicides reduced sclerotia germination when applied to soils in the fall and/or spring and several fungicides reduced ergot at harvest when applied to flowers during anthesis. These data could be used to enter new fungicides/application methods into the IR-4 program. A model was developed to predict ergot ascospore presence in Columbia Basin perennial ryegrass seed production fields. This model identified environmental factors that are associated with ergot ascospore production and can be included in the 2016 Ergot Alert Newsletter so growers can better evaluate ergot risk in their fields. Cultivars of Kentucky bluegrass (KBG) and perennial ryegrass (PRG) that resist/escape ergot infection were identified and ergot levels were correlated with anthesis initiation and duration. This information could be used by breeders to develop cultivars with reduced ergot. Differences in disease incidence were not observed among micro-plots spatially separated 20 ft. away from a honeydew-laden plant, suggesting that honeydew can be dispersed at least this far via water or insects. Commercial fields were surveyed to study relationships between insect populations and ergot levels. This study will provide important information to increase our understanding of the importance and potential vectors of ergot honeydew.

We presented our findings at the Grass Seed Field Days held at HAREC/COARC and at various grower education meetings. 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. Two poster presentations were made at the annual American Phytopathological Society meeting in Pasadena, CA (August 2015). Additional extension publications and presentations will be made available to growers in the Pacific Northwest region in the future.

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TABLES AND FIGURES

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

Treatment	Chemical	Product	Manufacturer	Rate	Timing
01	Nontreated control	NA	NA	NA	Fall + Spring
02	Pyraclostrobin + fluxapyroxad	Priaxor	BASF	6 oz/acre	Spring
03	Cyproconazole	Alto	Syngenta	5.5 oz/acre	Spring
04	Propiconazole	Tilt	Syngenta	8 oz/acre	Spring
05	Prothioconazole	Proline	Bayer CS	5.7 oz/acre	Spring
06	Fluopyram	Luna	Bayer CS	5.5 oz/acre	Spring
07	Benzovindiflupyr	Solatenol	Syngenta	50 grams a.i/ha	Spring
08	Penthiopyrad	Fontelis	DuPont	24 oz/acre	Spring
09	Penthiopyrad	Fontelis	DuPont	24 oz/acre	Fall
10	Fluopyram + prothioconazole	Propulse	Bayer CS	14 oz/acre	Fall
11	Fluopyram + prothioconazole	Propulse	Bayer CS	14 oz/acre	Spring
12	Fluopyram + prothioconazole	Propulse	Bayer CS	14 oz/acre	Fall + Spring
13	Azoxystrobin + propiconazole	Quilt Xcel	Syngenta	14 oz/acre	Fall
14	Azoxystrobin + propiconazole	Quilt Xcel	Syngenta	14 oz/acre	Spring
15	Azoxystrobin + propiconazole	Quilt Xcel	Syngenta	14 oz/acre	Fall + Spring
16	Pyraclostrobin	Headline	BASF	12 oz/acre	Fall
17	Pyraclostrobin	Headline	BASF	12 oz/acre	Spring
18	Pyraclostrobin	Headline	BASF	12 oz/acre	Fall + Spring
19	Picoxystrobin	Aproach	DuPont	18 oz/acre	Fall
20	Picoxystrobin	Aproach	DuPont	18 oz/acre	Spring
21	Picoxystrobin	Aproach	DuPont	18 oz/acre	Fall + Spring
22	Azoxystrobin	Abound	Syngenta	15.5 oz/acre	Fall
23	Azoxystrobin	Abound	Syngenta	15.5 oz/acre	Spring
24	Azoxystrobin	Abound	Syngenta	15.5 oz/acre	Fall + Spring

Table 2. Burkard spore trap monitoring site, location, species, cultivar, and planting date of the crop.

Site	County	Grass species	Cultivar	Planting date
PRG-1	Umatilla, OR	Perennial ryegrass	Multiple (cultivar trial)	Aug 29 2014
PRG-2	Umatilla, OR	Perennial ryegrass	Pavilion	Sept 20 2013
KBG-1	Benton, WA	Kentucky bluegrass	Arrowhead	Sept 2 2014
KBG-2	Benton, WA	Kentucky bluegrass	Arrowhead	Sept 3 2014
KBG-3	Union, OR	Kentucky bluegrass	Wildhorse	May 5 2010
KBG-4	Union, OR	Kentucky bluegrass	Baron	April/May 2014
KBG-5	Jefferson, OR	Kentucky bluegrass	Multiple (cultivar trial)	Aug 11 2014

Table 3. Area under sclerotia germination curve (AUSGC) and area under capitula production curve (AUCPC) values following fungicide treatments when soil moisture was either dry or wet

Fungicide	Soil moisture	AUSGC	AUCPC
Control	**Dry**	293 a	461 a
Control	**Wet**	281 a	442 a
Azoxystrobin	**Dry**	194 a	286 ab
Azoxystrobin	**Wet**	290 a	435 a
Picoxystrobin + Cyproconazole	**Dry**	67 bc	95 b
Picoxystrobin + Cyproconazole	**Wet**	39 c	47 b
Pyraclostrobin	**Dry**	194 ab	252 ab
Pyraclostrobin	**Wet**	253 a	297 a

Table 4. Area under capitula production curve (AUCPC) values following fungicide treatments on germinated sclerotia

Treatment	No. of germinated sclerotia	AUCPC
Control	12.8	83
Penthiopyrad	14.3	77
Tetraconazole	15.0	80
Azoxystrobin + propiconazole	16.0	78
Benzovindiflupyr	15.3	86
Cyflufenamid	12.3	76
Zoxamide+Chlorothalonil	14.7	78

Table 5. Correlations (*r*-values) between the number of ergot spores captured in 2015 and environmental data collected from Watchdog data loggers placed in the field or from the AgriMet MRSO weather station located at COARC[1]

Environmental variable	Field	MRSO
Maximum air temperature	0.38*	0.40*
Minimum air temperature	0.50*	0.52*
Mean air temperature	0.46*	0.47*
Air daily degree days	0.25*	0.46*
Air cumulative degree days	0.33*	0.32*
Maximum soil temperature	-0.02	0.52*
Minimum soil temperature	0.48*	0.47*
Mean soil temperature	0.36*	0.49*
Soil daily degree days	0.30*	0.51*
Soil cumulative degree days	0.35*	0.32*
Relative humidity	0.17	0.04
Dew point	0.52*	0.51*
Soil moisture	0.48*	NR[2]
Daily precipitation	NR	-0.11
Evapotranspiration	NR	0.37*

[1] An *r*-value = 1 indicates a perfect correlation, while an *r*-value = 0 indicates no correlation. A * indicates the correlation was significant at $P < 0.05$.
[2] Not recorded

Table 6. Accuracy of environmental favorability index (EFI) model used to predict the occurrence of at least one ergot ascospore in 2013, 2014, 2015, and all three years combined

Environmental Favorability Index (EFI)[1]	2013			2014			2015			2013-2015 Combined		
	Correct predictions	False positives	False negatives	Correct predictions	False positives	False negatives	Correct predictions	False positives	False negatives	Correct predictions	False positives	False negatives
EFI ≥ 1	80.9%	13.8%	5.3%	73.0%	25.6%	1.4%	64.2%	25.3%	10.5%	72.5%	21.5%	6.0%
EFI ≥ 2	88.2%	0.0%	11.8%	78.7%	15.6%	5.7%	75.3%	6.2%	18.5%	80.7%	7.0%	12.3%
EFI ≥ 3	77.0%	0.0%	23.0%	76.6%	13.5%	9.9%	70.4%	3.7%	25.9%	74.5%	5.5%	20.0%
EFI ≥ 4	65.8%	0.0%	34.2%	61.0%	11.3%	27.7%	70.4%	1.9%	27.7%	65.9%	4.2%	29.9%
EFI ≥ 5	52.6%	0.0%	47.4%	53.9%	9.9%	36.2%	55.6%	0.6%	43.8%	54.1%	3.3%	42.6%

[1] Environmental variables used in the environmental favorability index (EFI) model included maximum, minimum, and mean soil temperature, daily soil degree days, and mean dew point.

Table. 7. Anthesis timing, total spores during anthesis, ergot incidence, and ergot severity of twelve Kentucky bluegrass cultivars evaluated for ergot infection[1]

Treatment	Anthesis_ initiation	Anthesis_ termination	Anthesis_ duration	Total spores during anthesis	Incidence (%)[2]	Severity[2]
Midnight II	143.8 a	162.8 ab	19.0 ab	686 ab	17 a	51.8 a
Gladstone	134.8 d	159.0 abc	24.3 ab	511 abc	11 ab	35.3 ab
Blue Ghost	136.0 c	153.0 bc	17.0 b	229 c	11 ab	27.5 ab
Nuglade	143.5 b	164.5 ab	21.0 ab	811 a	10 ab	26.3 ab
Bluechip	134.5 d	161.0 ab	26.5 a	605 b	10 ab	23.0 ab
DB-1013	135.8 d	153.0 bc	17.3 b	229 c	7 ab	14.0 b
Gateway	141.0 b	159.0 abc	18.0 b	511 abc	8 ab	11.0 b
Shamrock	134.8 d	153.0 bc	18.3 b	229 c	3 b	11.0 b
Right	135.3 d	153.0 bc	17.8 b	229 c	4 b	6.0 b
PST-K4-7	141.0 abc	161.0 ab	20.0 ab	605 ab	5 b	5.0 b
Jumpstart	136.0 d	155.0 bc	19.0 ab	323 bc	2 b	3.8 b
Fielder	135.3 d	155.0 bc	19.8 ab	323 bc	3 b	3.3 b
P-value	$P < 0.0001$	$P < 0.0001$	$P = 0.003$	$P < 0.0001$	$P = 0.0002$	$P = 0.0006$

[1] Means followed by the same letters are not statistically different using Tukey's comparison. Dates are based on perpetual Julian days (134 = May 14; 164 = June 12).
[2] Disease incidence (number of infected seed heads) and severity (number of sclerotia) were determined from a random sample of 100 seed heads collected from each plot at harvest.

Table. 8. Anthesis timing, total spores during anthesis, ergot incidence, and ergot severity of the twelve perennial ryegrass cultivars evaluated for ergot infection[1]

Treatment	Anthesis_ initiation	Anthesis_mid	Anthesis_ termination	Total spores during anthesis	Incidence[2] (%)	Severity[2]
Applaud II	128.7 ab	144 ab	161 bc	574.5	38.8 bc	7.8
1G2	136.5 a	150.2 a	165 abc	422.5	18.8 bcd	4.5
Integra II	133 ab	150.2 a	169.5 ab	458.0	16.7 cd	6.3
PST-2M20	135.3 a	153.6 a	172.1 a	397.3	10.0 d	4.7
Silver Dollar	129 ab	135.1 bc	165 abc	577.3	46.7 ab	11.7
Quickstart II	124.5 b	132 c	158.5c	755.5	63.8 a	9.2
TopHat2	138.3 a	149.5 ab	161.8 bc	340.0	51.6 d	2.3
Derby Extreme	138 a	149.2 ab	168.8 ab	317.3	11.7 d	3.3
Esquire	135.5 a	151.5 a	168.2 ab	393.0	45.0 abcd	13.0
Fiesta4	130.7 ab	146 ab	166.2 ab	508.5	32.5 bcd	6.8
SR4600	134 ab	148.1 ab	160.1 bc	428.7	45.0 abc	7.7
Karma	136 a	150.4 a	165.6 abc	364.0	18.3 bcd	2.0
P-value	0.0009	<0.001	<0.0002	0.07 (NS)	<0.001	0.48 (NS)

[1] Means followed by the same letters are not statistically different using Tukey's comparison. Dates are based on perpetual Julian days (124 = May 4; 172 = June 21). [2] Disease incidence (number of infected seed heads) and severity (number of sclerotia) were determined from a random sample of 100 seed heads collected from each plot at harvest.

Table 9. Ergot incidence and severity in perennial ryegrass micro-plots located 3.3 ft. to 19.7 ft. away from a point source of honeydew inoculum.

Distance from honeydew (ft.)	Incidence[2]	Severity[2]
3.3	0.16	0.15
6.6	0.13	0.12
9.8	0.22	0.24
13.1	0.21	0.19
16.4	0.14	0.14
19.7	0.13	0.13
χ^2 value	NS	NS

[2] Disease incidence (number of infected seed heads) and severity (number of sclerotia) were determined from a random sample of 100 seed heads collected from each plot at harvest.

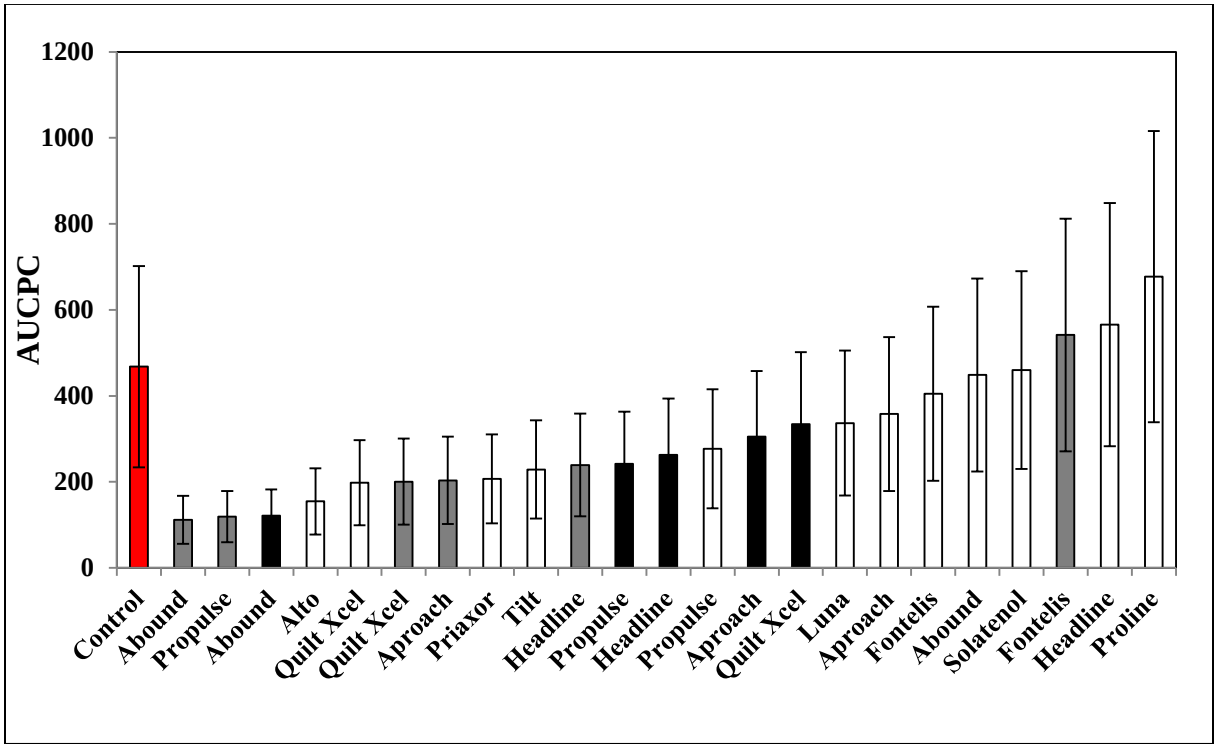

Fig. 2. Mean area under capitula production curve (AUCPC) values in experimental plots infested with ergot sclerotia from perennial ryegrass in October 2014 and treated with soil-applied fungicides in fall 2014 (gray bars), spring 2015 (white bars), or in both fall 2014 and spring 2015 (black bars). The red bar represents the water-treated control.

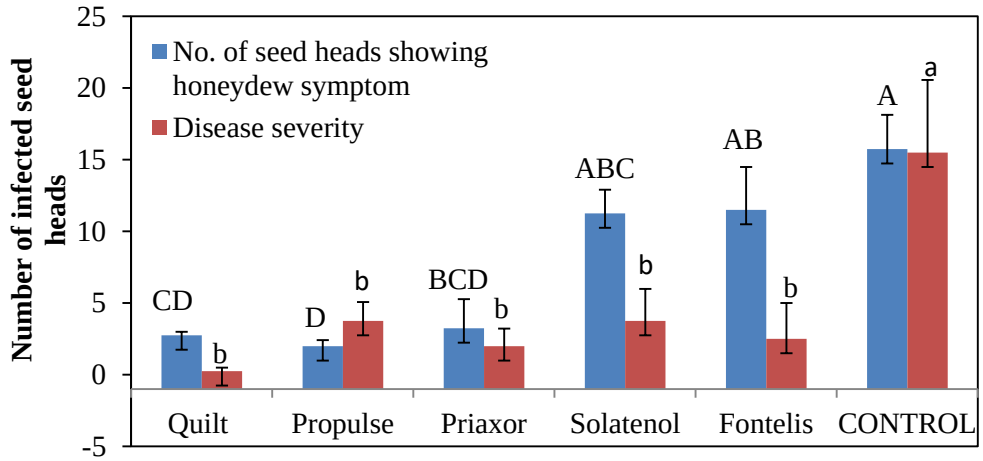

Fig. 3. Effect of anthesis applications of fungicide treatments on ergot infection in PRG.

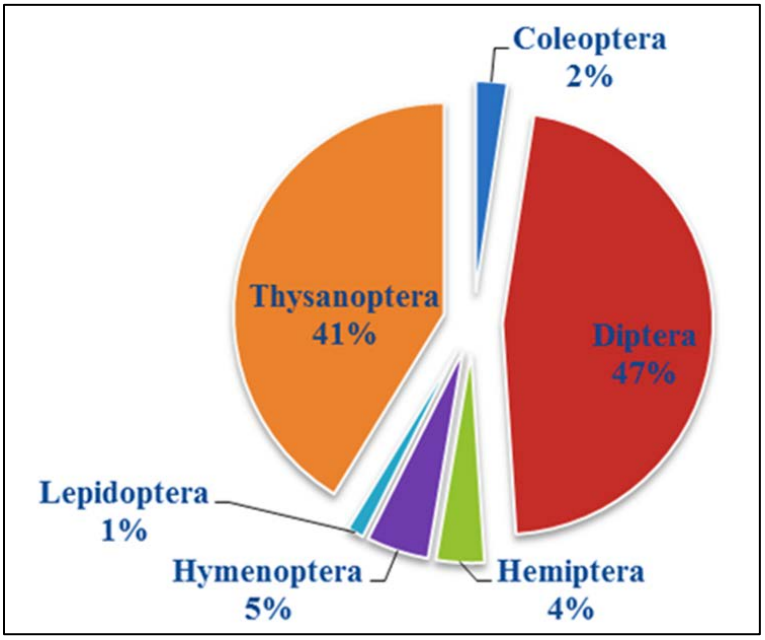

Fig. 4. Relative abundance of insect groups collected from commercial grass seed fields from April to June 2015.

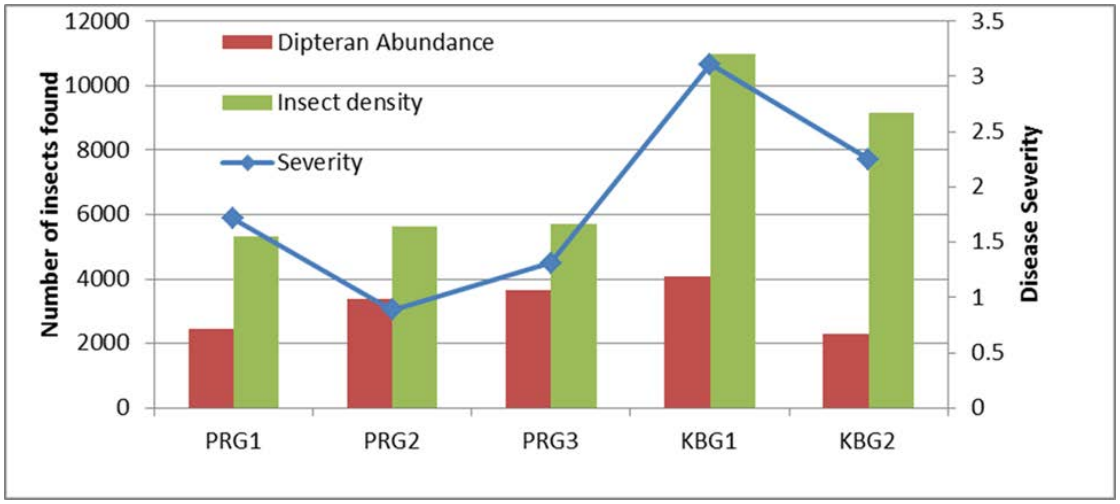

Fig. 5. Association between insect abundance and disease severity in commercial fields sampled from April to June 2015.

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