WASHINGTON TURFGRASS SEED COMMISSION
PROGRESS REPORT FOR 2019 PROJECTS

Project No.:_____

Title: Integrated Disease Management of Ergot in Kentucky Bluegrass

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Cooperators: Jory Iverson, Mike Hawman

Reporting Period: July 2019-November 2019

Accomplishments:
Our first objective was to validate a crowdsourced Ergot Alert Network that will enlist grass seed stakeholders in participatory research, disease monitoring, and predictive model development. For the first year of validation, 238 data points were collected and analyzed. Overall, 91.6% of the data points agreed between rotating-arm spore samplers and the standard Burkard spore samplers. Sampling height was not a significant factor affecting sampling efficiency, but a minimum sampling period of at least 2 days is recommended. This research suggests that the rotating-arm spore samplers can perform as well as the standard Burkard spore samplers for monitoring airborne ergot ascospores. These results will be further validated in the second year. *Our second objective was to screen novel fungicide chemistries, alone and in combination, for ergot control during anthesis.* Eight treatments were compared to an industry standard treatment and a non-treated control at field plots of perennial ryegrass in Hermiston and Kentucky bluegrass in Madras. This research contributes towards the development of comprehensive integrated disease management strategies for ergot in grass seed crops of the Pacific Northwest.

Results:
Objective 1: A total of 238 sampling events were performed during the first year of validation and ergot spores were detected in 211 (88.7%) of the sampling events. Results from the rotating-arm samplers were consistent with results from the Burkard spore sampler for 91.6% of the data points. There were no statistical difference among collection heights and Burkard sampler (P = 0.15), indicating that rotating-arm samplers performed equally at different collection heights and were comparable to the standard Burkard spore sampler. There was a significant difference among sampling period ($P < 0.0001$) and ergot detection was greatest in samples collected every 7 days, but the 2-day sampling period was not significantly different from the 7-day sampling period. Overall, rotating-arm spore samplers performed as well as Burkard spore samplers under wet conditions regardless of sampling height ($P = 0.32$). Samples collected every 7 days also performed best during precipitation events but were not significantly different from samples collected after 2 days, which corresponds to the results collected under all weather conditions.
Objective 2: Although the presence of inoculum was confirmed during anthesis at field plots in Hermiston and Madras, ergot incidence and severity was low in field plots at both locations. Consequently, a significant effect of fungicide treatment was not detected in either trial ($P > 0.05$).

Publications:

Kaur, N., Cating, R.A., Rondon, S.I., Scott, J.C., Alderman, S.C., Walenta, D.L., Frost, K.E., Hamm, P.B, and Dung, J.K.S.. 2019. Dispersal potential of ergot spores by insects foraging in perennial ryegrass fields in the Columbia Basin of Oregon and Washington. Crop, Forage, and Turfgrass Management. doi:10.2134/cftm2019.04.0020.

Cheng, Q., Dung, J.K.S., and Frost, K.E. 2019. Evaluation of fungicides for control of ergot on Kentucky bluegrass in Oregon, 2018. Plant Disease Management Reports 13:T001. doi: 10.1094/PDMR13.

WASHINGTON TURFGRASS SEED COMMISSION PROGRESS REPORT
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Instructions:

1. Record information for active and pending projects.
2. All current research to which principal investigator(s) and other senior personnel have committed a portion of their time must be listed whether or not salary for the person(s) involved is included in the budgets of the various projects.
3. Provide analogous information for all proposed research which is being considered by, or which will be submitted in the near future to, other possible sponsors.

Name (List PI#1 first)	Supporting Agency and Project #	Total $ Amount	Effective and Expiration Dates	% of Time Committed	Title of Project
	Current:				
Dung	California Fresh Carrot Advisory Board	$8,934	March 2019 – February 2020	1%	Evaluation of Copper Products for Bacterial Blight Control in Carrots
Dung	USDA-NIFA Western IPM Center	$29,747	March 2019 – February 2020	1%	Aerobiology of *Xanthomonas hortorum* pv. *carotae* in Carrot Seed Fields
Sullivan, Dung	Oregon Mint Commission	$14,959	July 2019 - June 2020	0.5%	Evaluating the Efficacy of Cover Crops to Control Verticillium Wilt, Reduce Mint-Pathogenic Nematodes, and Improve Soil Quality for Mint Production
Vining, Dung	Oregon Mint Commission	$23,658	July 2019 - June 2020	0.5%	Development of a Diagnostic Quantitative PCR Assay for Detection of *Verticillium dahliae* in Mint
Dung, Sagili	Oregon Agricultural Research Foundation	$12,483	March 2019 – February 2021	1%	Can Honey Bees Serve as Vectors of Bacterial Blight in Carrot Seed Crops of Central Oregon?
Dung	Mint Industry Research Council	$28,462	July 2019 – June 2020	1%	Identifying Economic Action Thresholds to Inform Verticillium Wilt Management Decisions
Lange, Vining, Dung	Mint Industry Research Council	$37,660	July 2019 - June 2020	0.5%	Verticillium Control Through Natural Antifungal Terpenoids

Pappu et al.	USDA-NIFA SCRI	$3,291,766	September 2018 – August 2022	4%	Managing Stakeholder-Prioritized Pests and Diseases Threatening the US *Allium* Industry
Dung	Oregon Agricultural Research Foundation	$12,500	March 2018 – February 2020	1%	Epidemiology of *Xanthomonas hortorum* pv. *carotae* in Carrot Seed Fields
Weiland, Dung	USDA-ARS CRIS	$142,200	September 2015 – September 2020	0.5%	Identification, Detection, and Diagnostics of *Verticillium* Species of the Pacific Northwest
Dung, Frost, Cheng, Walenta,	Washington Turfgrass Seed Commission	$26,000	July 2019 - June 2020	1%	Integrated Disease Management of Ergot in Kentucky Bluegrass
Dung, Frost, Cheng, Walenta	Columbia Basin Grass Seed Growers Association	$10,000	July 2019 - June 2020	1%	Understanding the Biology and Improving Control of Ergot in Grass Grown for Seed
Dung, Frost, Cheng, Walenta	Oregon Seed Council	$10,000	July 2019 - June 2020	1%	Crowd-Sourcing Disease Detection Networks for Enhanced IPM in Grass Seed Crops
Goyer, Rondon, DeBano, Wooster, Frost, Sathuvalli	USDA-NIFA-NNF	$246,000	8/1/16 – 12/31/19	1%	Enhancing professional quality of future leaders in agriculture and natural resources: new strategies for graduate student training
Frost, Dung	USDA-NIFA-CARE	$294,000	04/1/17 – 12/31/19	5%	Integrating grower maintained and publically held data to improve potato early dying management
Charkowski, et al.	USDA-NIFA-SCRI	$2.5M	10/1/17 – 9/30/22	4%	Integrating next-generation technologies for blackleg and soft rot management in potato

Charkowski et al.	Farm Bill 10007 (USDA APHIS PPQ S&T)	$320,700	7/1/19 – 6/30/20	1%	Safeguarding the US seed potato industry against emerging seed potato-borne pathogens that impact trade and farm viability
Rosen, et al.	USDA-SCRI	$8.1M	9/1/18 – 8/31/22	4%	Enhancing soil health in U.S. potato production systems
Frost, Huseth, Anderson, Groves, Cooper	USDA-ARS State Partnership Program	$60,000	7/1/18-6/30/20	1%	Potato cultivar sensitivity to feeding of three Lygus species
Rosenzweig, Frost, Hao, Larkin	USDA-ARS State Partnership Program	$60,000	7/1/18-6/30/20	1%	Fungicide resistance monitoring of *Helminthosporium solani* isolates from potatoes to determine population sensitivity distribution, risk assessment, and gene mutations associated with resistance
Duellman, Wharton, Woodhall, Frost, Inglis, McMoran	Northwest Potato Research Consortium	$40,906	7/1/19 – 6/30/20	1%	Characterizing Fusarium species associated with and refining management of potato dry rot in the Pacific Northwest
Frost	Northwest Potato Research Consortium	$38,940	7/1/19 – 6/30/20	1%	Using next generation sequencing to characterize the total microbial community in soils associated with seed potato
Frost	Northwest Potato Research Consortium	$14,321	7/1/19 – 6/30/20	1%	Development of a web-based late blight forecasting application
Frost	Northwest Potato Research Consortium	$32,673	7/1/19 – 6/30/20	1%	Data mining for crop rotations that predict the occurrence of mefanoxam-resistant Pythium species

Karasev, Delparte, Frost	Northwest Potato Research Consortium	$6,945	7/1/19 – 6/30/20	1%	Monitoring PVY strains in the Othello and Hermiston trials
Cooper, Horton, Crowder, Frost	Northwest Potato Research Consortium	$7,993	7/1/19 – 6/30/20	1%	Molecular and landscape approaches to understanding beet leafhopper and potato purple top disease in the Columbia Basin
Frost	Oregon Potato Commission	$20,800	7/1/19 – 6/30/20	1%	Potato pathology seed lot trials and Extension program
Zasada, Gleason, MacGuidwin, Frost	USDA-ARS State Partnership Program	$55,393	7/1/19 – 6/30/20	1%	Nematode community assessment as part of defining soil health
Mollov, Hammerschmidt, Rosenzweig, Kinkle, Frost	USDA-ARS State Partnership Program	$125,000	7/1/19 – 6/30/20	1%	Developing potato common scab management strategies based on *Streptomyces* spp. diversity from suppressive and non-suppressive sites
Dung, Frost, Cheng, Walenta	Washington Turfgrass Seed Commission	$26,000	7/1/19 – 6/30/20	1%	Integrated disease management of ergot in Kentucky bluegrass
Dung, Frost, Cheng, Walenta	Oregon Seed Council	$10,000	7/1/19 – 6/30/20	1%	Crowd-sourcing disease detection networks for enhanced IPM in grass seed crops
	Pending:				
Dung et al.	USDA-NIFA SCRI	$3,000,000	September 2020-August 2024	5%	A Systems Approach for Managing Bacterial Blight of Carrot
Dung, Frost, Cheng, Walenta	Washington Turfgrass Seed Commission	$26,000	July 2020 – June 2021	1%	Controlling Ergot in Kentucky Bluegrass (*this proposal*)

Dung	Oregon Agricultural Research Foundation	$15,000	March 2020 – February 2022	1%	Development of a field-deployable assay for detecting ergot spores in grass seed production systems
Savory, Chang, Dung	Farm Bill 10007 FY19	$154,489	July 2020-June 2021	1%	Leveraging genomic resources to improve detection methods for *Xanthomonas hortorum* pv. *carotae* in Oregon
Dung	California Garlic and Onion Research Advisory Board	$7,700		1%	Genetic diversity and pathogenicity of *Fusarium proliferatum* causing clove rot on garlic
Hagerty, Mundt, Frost, Barroso, Zemetra, Machado	USDA-AFRI	$500,000	07/01/20 – 06/30/22	1%	Exploiting plant architecture to reduce biotic and abiotic stresses
Frost, Rosenzweig, Hammerschmidt	USDA-AFRI	$499,999	01/1/20 – 12/31/22	1%	Assessing and mitigating risk for development of tuber blemish diseases in potato production
Karasev et al.	USDA-SCRI	$5.7M	9/1/20 – 8/31/24	1%	Development of sustainable system-based management strategies for vector-borne, tuber necrotic viruses in potato

APPENDIX

OBJECTIVE 1:

HYPOTHESIS & OBJECTIVES:

The Ergot Alert Network is limited in the scope and number of Ergot Alert locations that can be monitored due to equipment costs, labor, and time restrictions. We propose to use a citizen science approach to overcome several logistical (travel, labor, and time) and technical (equipment, expertise) limitations that exist which hinder our team's ability to expand spore trapping beyond its current scope. The objective of this three-year project is to validate (Years 1 and 2) and establish (Year 3) a crowdsourced Ergot Alert Network that will enlist grass seed stakeholders in participatory research, disease monitoring, and predictive model development.

PROCEDURES:

Year 1 - Validation: Instead of Burkard spore samplers, which are expensive (>$5,000) and difficult to maintain, we deployed and validated rotating-arm samplers which were relatively inexpensive (< $100) and easy to build and maintain. Validation of the rotating-arm samplers was performed at COAREC (Madras, OR) in a second year, five-acre Kentucky bluegrass seed field with a history of ergot. Three plots were established in the five-acre field for the validation. In each of the plots, one Burkard spore sampler was placed alongside three rotating-arm samplers to collect ergot spores simultaneously (Fig 1). The three rotating-arm samplers in each plot were set at different heights (2ft, 3ft and 4ft) in order to test the effect of sampling height on spore collection efficiency. Different sampling periods (1d, 2d, 3d, 4d, and 7d) were also tested and compared with the standard Burkard spore sampler. Samples were processed following the standard phenol-chloroform DNA extraction procedure and quantified by quantitative PCR (qPCR) (Dung et al. 2018). Precipitation data were collected to determine the performance of the rotating-arm samplers under wet conditions. The collection efficiencies of traps with different heights and sampling periods were compared using analysis of variance. Results from Burkard samplers were provided through the Ergot Alert Blog (http://blogs.oregonstate.edu/coarecplantpathology/) as in previous years.

RESULTS AND DISCUSSION

A total of 238 sampling events were performed during the first year of validation (Table 1). Ergot spores were detected in 211 (88.7%) of the sampling events (Table 2). Results from the rotating-arm samplers were consistent with results from the Burkard spore sampler for 91.6% of the data points. False negatives, which were defined as days on which ergot spores were not detected by an individual sampler but were detected by at least one other sampler in the array during the same sampling period, were highest in the 4ft rotating-arm sampler and Burkard spore sampler (7 false negatives), followed by the 2ft rotating-arm sampler (4 false negatives) and the 3ft rotating-arm sampler (2 false negatives) (Table 2).

Rotating-arm samplers set at collection heights of 2ft, 3ft, and 4ft were compared with the standard Burkard spore sampler, which collects at a height of 2ft. There were no statistical difference among collection heights and Burkard sampler ($P = 0.15$) (Table 3), indicating that rotating-arm samplers performed equally at different collection heights and were comparable to the standard Burkard spore sampler. It was noted that as the season progressed, operation of the rotating-arm samplers at 2ft heights were compromised by the canopy, so it will be

recommended that the rotating-arm samplers are placed just above the expected canopy height at anthesis.

Different sampling periods (1d, 2d, 3d, 4d, and 7d) were tested and compared with the standard Burkard spore sampler. There was a significant difference among sampling period ($P < 0.0001$) (Table 4). Overall, ergot detection was greatest in samples collected every 7 days, but the 2-day sampling period was not significantly different from the 7-day sampling period (Table 4).

Precipitation events were recorded and data for sampling periods with precipitation events were analyzed separately in order to validate the performance of spore samplers in wet conditions. Overall, rotating-arm spore samplers performed as well as Burkard spore samplers under wet conditions regardless of sampling height ($P = 0.32$) (Table 5). Samples collected every 7 days also performed best during precipitation events but were not significantly different than samples collected after 2 days (Table 6), which corresponds to the results collected under all weather conditions.

SUMMARY:

For the first year of validation, 238 data points were collected and analyzed. Overall, 91.6% of the data points agreed between rotating-arm spore samplers and the standard Burkard spore samplers. Sampling height was not a significant factor affecting sampling efficiency, but a minimum sampling period of at least 2 days is recommended. A rotating-arm spore sampler was also deployed in the Grande Ronde Valley as a preliminary test of the new system in a commercial KBG seed production field. This research suggests that the rotating-arm spore samplers can perform as well as the standard Burkard spore samplers for monitoring airborne ergot ascospores. These results will be further validated in the second year. This research contributes towards the development of comprehensive integrated disease management strategies for ergot in grass seed crops of Oregon and the Pacific Northwest.

ACKNOWLEDGEMENTS:

The technical support provided by Victoria Skillman, Hoyt Downing, and Mitchell Alley was greatly appreciated. Additional funding was provided by the Washington Turfgrass Seed Commission, the Oregon Seed Council/ODA Alternatives for Field Burning Research Financial Assistance Program, the Columbia Basin Grass Seed Association, and the Jefferson County Grass Seed Growers Association.

TABLES AND FIGURES:

Fig 1. Rotating-arm spore samplers at different sampling heights (2ft, 3ft, and 4ft above the ground) were placed alongside a Burkard spore sampler to collect ergot spores simultaneously in the field.

Table 1. Cycle threshold values for the different rotating-arm sampling periods and heights and compared with a Burkard spore sampler.[1]

	Sampling Period (# of samples)				
Sampling Height	**1d (*n*=11)**	**2d (*n*=24)**	**3d (*n*=119)**	**4d (*n*=60)**	**7d (*n*=24)**
Rotating-Arm (2ft)	35.13	23.95	29.13	28.39	24.57
Rotating-Arm (3ft)	31.83	25.51	28.71	28.91	26.22
Rotating-Arm (4ft)	40.00	27.34	29.35	28.82	26.17
Burkard (2ft)	34.03	26.13	29.94	26.52	24.19
***P*-value**	**0.06**	**0.07**	**0.79**	**0.22**	**0.12**

[1] A smaller cycle threshold value indicates that more spores were collected.

Table 2. Number of days on which ergot spores were detected or not detected using different rotating-arm sampling periods and heights and compared with a Burkard spore sampler.

Sampling Period	Sampling Height	Detected	Not Detected	False Negatives[1]
1 day	**Rotating-Arm (2ft)**	2	1	0
	Rotating-Arm (3ft)	2	1	0
	Rotating-Arm (4ft)	0	0	2
	Burkard (2ft)	1	1	1
2 days	**Rotating-Arm (2ft)**	6	0	0
	Rotating-Arm (3ft)	6	0	0
	Rotating-Arm (4ft)	6	0	0
	Burkard (2ft)	6	0	0
3 days	**Rotating-Arm (2ft)**	26	1	3
	Rotating-Arm (3ft)	28	1	1
	Rotating-Arm (4ft)	25	1	3
	Burkard (2ft)	23	1	6
4 days	**Rotating-Arm (2ft)**	14	0	1
	Rotating-Arm (3ft)	14	0	1
	Rotating-Arm (4ft)	13	0	2
	Burkard (2ft)	15	0	0
7 days	**Rotating-Arm (2ft)**	6	0	0
	Rotating-Arm (3ft)	6	0	0
	Rotating-Arm (4ft)	6	0	0
	Burkard (2ft)	6	0	0

[1] False negatives were defined as days on which ergot spores were not detected by an individual sampler but were detected by at least one other sampler in the array during the same sampling period.

Table 3. Effect of different rotating-arm sampling heights (2ft, 3ft, and 4ft) on ergot spore collection and compared with a Burkard spore sampler.

Sampling Height	Mean Cycle Threshold Value[1]
Rotating-Arm (4ft) (*n*= 58)	30.33
Rotating-Arm (3ft) (*n*= 60)	28.24
Rotating-Arm (2ft) (*n*= 60)	28.23
Burkard (2ft) (*n*= 60)	28.16
***P*-value**	**0.14**

[1] A smaller cycle threshold value indicates that more spores were collected.

Table 4. Effects of different rotating-arm sampling periods (1d, 2d, 3d, 4d, and 7d) on ergot spore collection.

Sampling Period (number of samples)	**Mean Cycle Threshold Value**[1]
1d (*n*=11)	35.25 a
3d (*n*=119)	29.28 b
4d (*n*=60)	28.16 bc
2d (*n*=24)	25.73 cd
7d (*n*=24)	25.29 d
***P*- value**	**<0.0001**

[1] A smaller cycle threshold value indicates that more spores were collected. Treatments followed by the same letters are not significantly different from each other using Tukey's test.

Table 5. Effect of different rotating-arm sampling heights (2ft, 3ft, and 4ft) on ergot spore collection during precipitation events and compared with a Burkard spore sampler.

Sampling Height	**Mean Cycle Threshold Value**[1]
Rotating-Arm (4ft) (*n*=31)	30.81
Rotating-Arm (3ft) (*n*= 33)	29.00
Burkard (2ft) (*n*= 33)	28.91
Rotating-Arm (2ft) (*n*= 33)	28.80
***P*-value**	**0.27**

[1] A smaller cycle threshold value indicates that more spores were collected.

Table 6. Effects of different rotating-arm sampling periods (1d, 2d, 3d, 4d and 7d) on ergot spore collection during precipitation events.

Sampling Period (number of samples)	**Mean Cycle Threshold Value**[1]
1d (*n*=11)	35.25 a
4d (*n*=24)	30.49 b
3d (*n*=59)	29.95 b
2d (*n*=24)	25.73 c
7d (*n*=12)	25.48 c
***P*- value**	**< 0.0001**

[1] A smaller cycle threshold value indicates that more spores were collected. Treatments followed by the same letters are not significantly different from each other using Tukey's test.

OBJECTIVE 2:
HYPOTHESIS & OBJECTIVES:

Only two active ingredients (azoxystrobin and propiconazole) are labeled for ergot control in grass grown for seed, and we hypothesize that variation in sensitivity to these fungicides exists among *C. purpurea* populations. *Objective 1 is to determine the baseline sensitivity of C. purpurea populations to azoxystrobin and propiconazole, assess the potential for cross-resistance between azoxystrobin and propiconazole, and select a discriminatory concentration for routine sensitivity monitoring.* We also hypothesize that novel fungicide chemistries can be used to protect flowers from infection during flowering. *Objective 2 is to screen novel fungicide chemistries and tank-mixes for the ergot control during anthesis.*

PROCEDURES:

Objective 2a: Screening C. purpurea Isolates for Fungicide Sensitivity/Resistance

Based on their modes of action, resistance to QoI inhibitors such as azoxystrobin are typically measured by spore germination inhibition assays and resistance to DMI fungicides like propiconazole are typically measured by mycelial growth inhibition assays. In this study, we included both methodologies for each fungicide using potato dextrose agar (PDA) amended with technical grade azoxystrobin or propiconazole at a final concentration of 0.1, 1, 5, 10, and 50 µg/ml (ppm) in acetone. Salicylhydroxamic acid (SHAM) in methanol was added to media at a final concentration of 100 µg/ml to block the use of an alternate pathway for cellular respiration. Control plates were amended with acetone and SHAM, where concentrations of acetone and methanol were 1% vol/vol.

To measure the inhibitory effect of fungicides on mycelial growth, mycelial plugs (4 mm in diameter) of 50 *C. purpurea* isolates were excised from the edge of 14-day-old colonies and placed mycelia side down onto fungicide-amended PDA. Plates were maintained at 18 °C in the dark. Average diameters were measured in two perpendicular directions at 7- and 21-days post-plating and mycelial growth rates (cm/day) were calculated. Three replicate plates were used for each combination of isolate and fungicide concentration and the experiment was repeated once.

To measure the inhibitory effect of fungicides on spore germination, a conidial spore suspension (1×10^4 conidia/ml) of 10 *C. purpurea* isolates were prepared from a 10-day-old *C. purpurea* culture grown on *Claviceps* medium (Gilmore et al. 2016). A spore suspension of each isolate was spread evenly onto fungicide-amended PDA. Plates were incubated in dark at 18 °C, and percent germination was determined after 24 h based on 50 randomly-selected conidia. Three replicate plates were used for each combination of isolate and fungicide concentration and the experiment was repeated once.

The effective fungicide concentrations to reduce mycelial growth or spore germination by 50% (EC_{50}) were calculated in the R package "drc". A resistance factor, used to measure the resistance level of a population, was defined for each fungicide as the ratio of the least sensitive isolate's EC_{50} value to the median EC_{50} for that fungicide. Cross-resistance between the two fungicides was estimated using the EC_{50} values for each isolate to generate Spearman correlation coefficients for the pair of fungicides.

Objective 2b: Evaluation of Novel Fungicides for Ergot Control

Plots (20 × 3.5 ft.) of Kentucky bluegrass and perennial ryegrass were established in Madras and Hermiston, respectively, in August 2018. Plots were arranged in a randomized complete block design with 3 ft. buffer zones and replicated 5 times. Plots were artificially infested with ergot

sclerotia in October 2018. Treatments for ergot were applied during anthesis on June 2 (Hermiston) and June 8 (Madras). Eight treatments (Aproach, Aproach+Propimax, Badge SC, Fontelis, Miravis, Miravis Neo, Priaxor, and Trivapro) were compared to an industry standard treatment of Quilt Xcel and a non-treated control. Ergot incidence and severity were calculated based on the number of seed heads containing sclerotia and the number of sclerotia present, respectively, in 100 seed heads collected from each plot. Data were analyzed using ANOVA and multiple comparisons were made using Tukey's test in SAS.

RESULTS AND DISCUSSION:

Objective 2a: Screening C. purpurea Isolates for Fungicide Sensitivity/Resistance. Results for the two mycelial growth inhibition experiments were not significantly different (P = 0.17), so the results were combined. In the mycelial growth inhibition assay, 50 (100%) and 42 (84%) *C. purpurea* isolates demonstrated mycelial growth inhibition by 50% on the medium amended with propiconazole and azoxystrobin, respectively (Fig. 1). However, the calculated EC_{50} values for the 50 isolates ranged dramatically, from 0.018 to 0.46 ppm (median 0.18 ppm) for propiconazole and from 0.0014 to 751 ppm (median 7.29 ppm) for azoxystrobin (Fig 2; Table 1). Annual trends in propiconazole EC_{50} values were not observed (Fig. 3). The resistance factor for propiconazole and azoxystrobin was 2.6 and 99.2 in the mycelial growth assay, respectively.

Results for the two spore germination inhibition experiments were not significantly different (P = 0.60), so the results were combined. In the spore germination assay, 10 (100%) and 9 (90%) *C. purpurea* isolates exhibited 50% germination inhibition by azoxystrobin and propiconazole, respectively (Fig. 4). However, the isolates exhibited a range of EC_{50} values from 0.014 to 30.48 ppm (median 0.39 ppm) for azoxystrobin and from 0.024 to 97.4 ppm (median 2.53 ppm) for propiconazole (Table 1). The resistance factor in the spore germination assay for azoxystrobin and propiconazole was 78.2 and 38.5, respectively.

A significant correlation between the azoxystrobin spore germination assay and the propiconazole mycelial growth assay was not observed (P = 0.07), which suggests that cross-resistance is not occurring.

Objective 2b: Evaluation of Novel Fungicides for Ergot Control. Although the presence of inoculum was confirmed during anthesis at COAREC and HAREC, ergot incidence and severity was low in field plots at both locations. Consequently, a significant effect of fungicide treatment was not detected in either trial (P > 0.05) (Table 2).

SUMMARY:

Most of the isolates tested in this study were sensitive to azoxystrobin and propiconazole but a few isolates exhibited reduced sensitivity to one of either fungicides. Mycelia and conidia responded differently to azoxystrobin and propiconazole, which was expected based on the different modes of action of these two fungicides. Typically, the spore germination and mycelial growth inhibition assays are specifically performed for azoxystrobin and propiconazole, respectively, but the combination of these two testing methods can provide additional information regarding fungicide resistance of *C. purpurea* during infection (spore germination) and colonization (mycelial growth). The results generated from the study enable future monitoring efforts for fungicide sensitivity changes in *C. purpurea* populations causing ergot in grass seed crops.

ACKNOWLEDGEMENTS:

Additional funding for this research was provided by the Oregon Seed Council/ODA Alternatives for Field Burning Research Financial Assistance Program, the Columbia Basin Grass Seed Association, the Columbia Basin Grass Seed Association, and the Jefferson County Grass Seed Growers Association. The researchers would like to thank the following companies for providing in-kind support: BASF, Central Oregon Seeds, Inc., Columbia River Seed, Corteva Agriscience, Gowan USA, Syngenta, and Riverview Seed. The technical support provided by Tiffany Belvoir and Shaelynn Downing was greatly appreciated.

TABLES AND FIGURES:

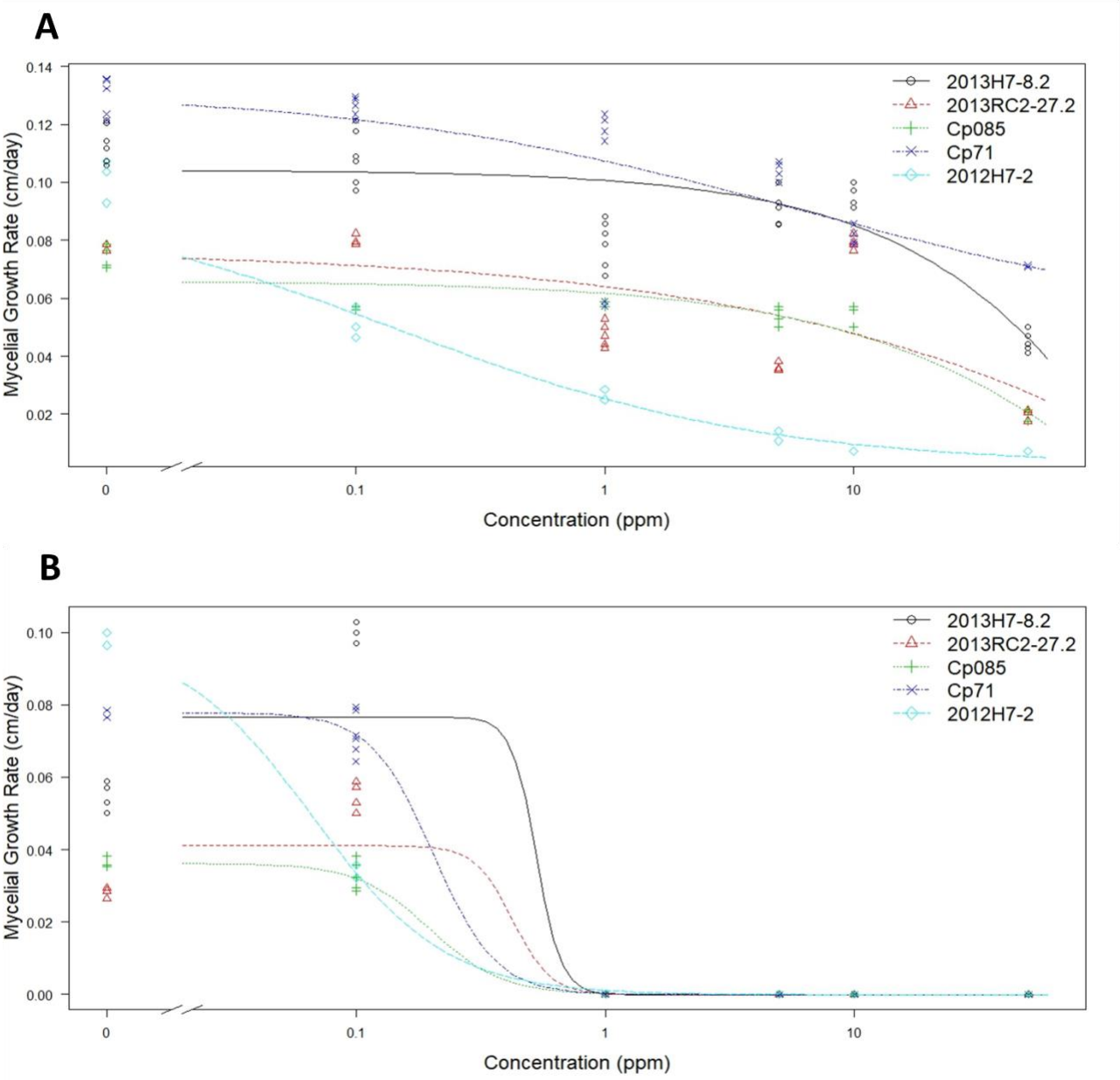

Fig 1. Mycelial growth rate of *Claviceps purpurea* isolates at different concentrations of azoxystrobin (A) and propiconazole (B) in a Petri plate assay.

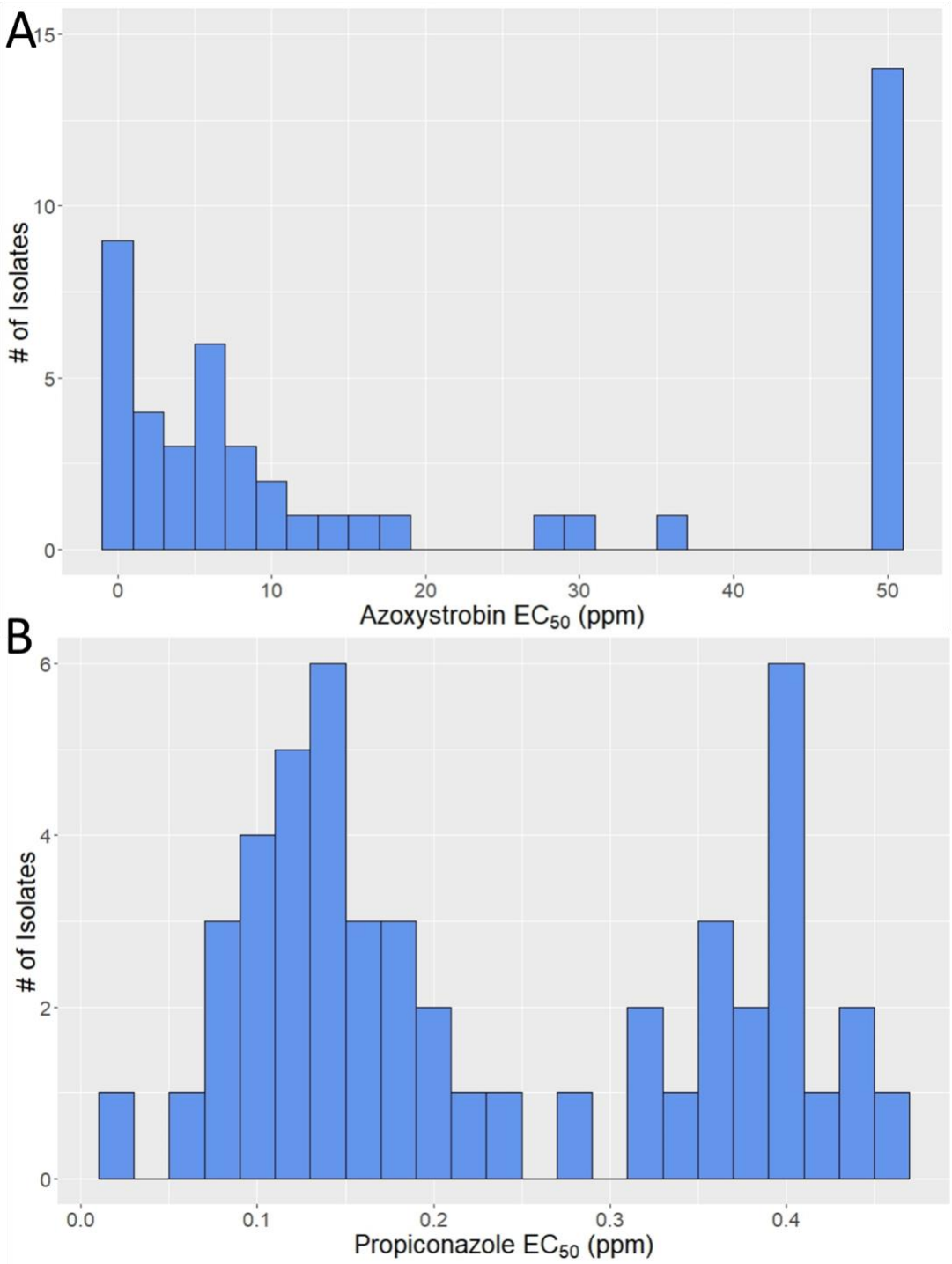

Fig 2. Frequency distribution (number of isolates) of effective azoxystrobin (A) and propiconazole (B) concentrations needed to inhibit the mycelial growth of *Claviceps purpurea* isolates by 50% (EC_{50}). Some EC_{50} larger than 50 ppm in (A) were binned into 50 in the histogram.

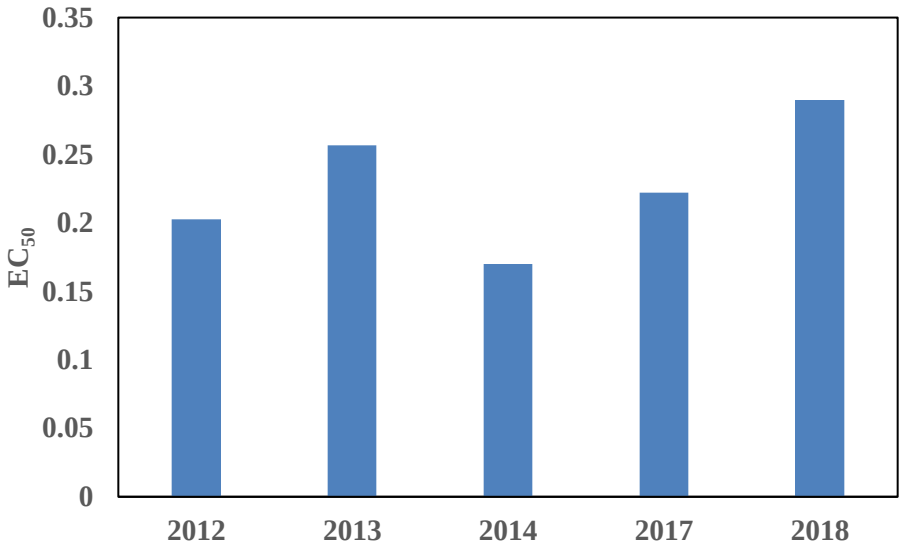

Fig. 3. Annual mean EC_{50} values for *Claviceps purpurea* isolates collected from Umatilla Co., OR. EC_{50} values were calculated using a mycelial growth inhibition assay.

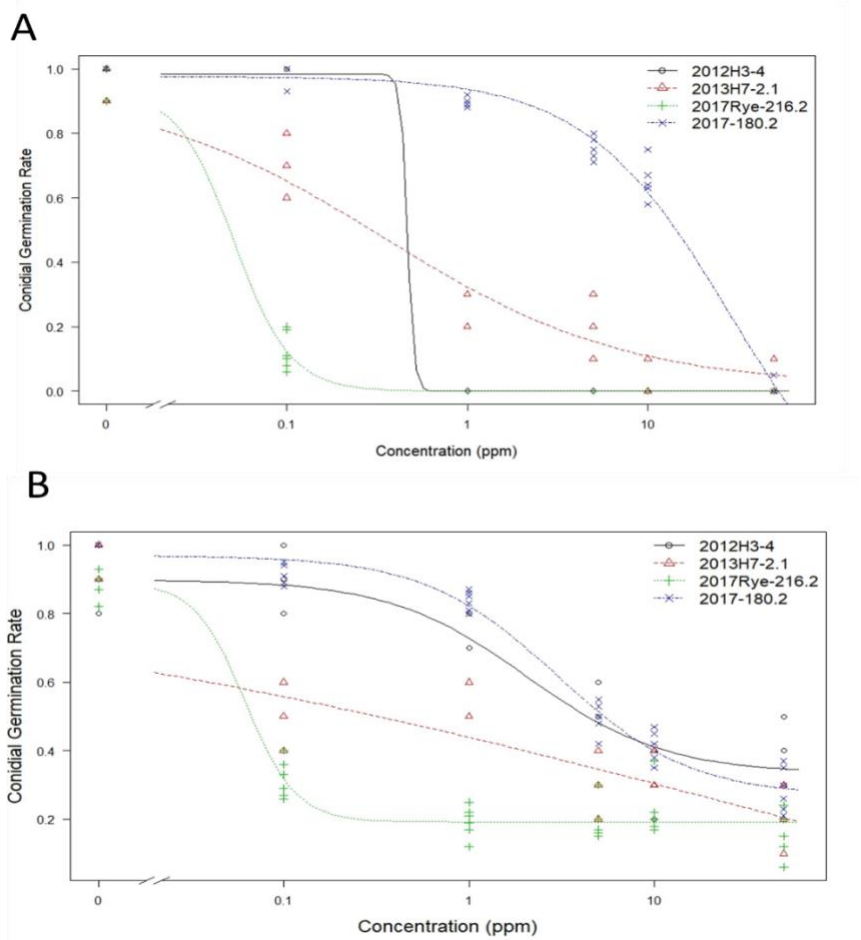

Fig 4. Conidia germination of *Claviceps purpurea* isolates at different concentrations of azoxystrobin (A) and propiconazole (B) in a Petri plate assay.

Table 1. Isolates used in this study and their corresponding host origin, year and location of isolation, and EC_{50} values from mycelium growth assays and conidia germination assays for azoxystrobin and propiconazole.

Host (Location)	Isolate	Year	Azoxystrobin EC_{50}		Propiconazole EC_{50}	
			Mycelia	Conidia	Mycelia	Conidia
Kentucky bluegrass (Jefferson Co., OR)	**KBG1**	**2010**	36.4	.	0.14	.
	KBG2	**2010**	115	.	0.28	.
	KBG3	**2015**	77.8	.	0.13	.
	KBG4	**2017**	0.032	.		.
	KBG5	**2017**	0.46	.	0.18	.
	KBG6	**2017**	0.76	.	0.11	28.5
	KBG7	**2017**	0.57	.	0.43	97.4
	KBG8	**2017**	17.4	.	0.14	.
	KBG9	**2018**	.	.	0.38	.
	KBG10	**2018**	145	.	0.33	.
Perennial ryegrass (Umatilla Co., OR)	**PRG1**	**2011**	16.24	.	0.37	.
	PRG2	**2012**	0.51	.	0.16	.
	PRG3	**2012**	0.0014	.	0.39	.
	PRG4	**2012**	0.19	.	0.1	.
	PRG5	**2012**	7.24	.	0.19	.
	PRG6	**2012**	7.84	.	0.13	.
	PRG7	**2012**	1.97	0.42	0.11	0.13
	PRG8	**2012**	.	0.41	0.41	2.05
	PRG9	**2012**	9.46	.	0.4	.
	PRG10	**2012**	27.2	0.022	0.18	0.024
	PRG11	**2012**	1.38	.	0.09	.
	PRG12	**2012**	0.14	.	0.07	.
	PRG13	**2013**	751	.	0.4	.
	PRG14	**2013**	126	.	0.23	.
	PRG15	**2013**	6.82	0.37	0.09	39.6
	PRG16	**2013**	1.15	.	0.37	.
	PRG17	**2013**	5.16	.	0.32	1.93
	PRG18	**2013**	10	.	0.45	.
	PRG19	**2013**	318	.	0.46	.
	PRG20	**2013**	1.34	.	0.36	3.19
	PRG21	**2013**	3.09	.	0.33	.
	PRG22	**2013**	0.7	.	0.15	.
	PRG23	**2013**	14.67	.	0.14	.

PRG24	**2013**	7.29	.	0.09	.
PRG25	**2013**	3.18	.	0.39	.
PRG26	**2013**	252	.	0.07	.
PRG27	**2013**	50.1	.	0.13	.
PRG28	**2013**	.	.	0.13	.
PRG29	**2014**	212	.	0.15	.
PRG30	**2014**	327	.	0.21	.
PRG31	**2014**	61.6	.	0.13	.
PRG32	**2014**	6.42	.	0.16	.
PRG33	**2014**	6.26	.	0.2	.
PRG34	**2017**	3.54	.	0.19	.
PRG35	**2017**	5.52	30.48	0.02	3.01
PRG36	**2017**	6.5	.	0.1	.
PRG37	**2017**	134	0.014	0.41	0.033
PRG38	**2017**	258	.	0.39	.
PRG39	**2018**	.	.	.	.
PRG40	**2018**	12.34	.	0.44	.
PRG41	**2018**	29.6	.	0.14	.

Table 2. Effect of fungicide applications at anthesis on ergot incidence and severity in Kentucky bluegrass and perennial ryegrass

		Kentucky bluegrass		**Perennial ryegrass**	
Treatment	**Rate (oz/acre)**	**Ergot incidence**	**Ergot severity**	**Ergot incidence**	**Ergot severity**
Aproach + Propimax	9.0 + 4.0	0.09	10.0	0.13	19.4
Aproach	9.0	0.07	6.7	0.06	4.6
Badge SC	34.6	0.01	1.0	0.06	4.4
Control	NA[1]	0.08	4.3	0.09	7.4
Fontelis	24.0	0.03	2.7	0.03	1.4
Miravis	3.8	0.05	2.3	0.05	4.0
Miravis Neo	13.7	0.01	0.3	0.02	1.4
Priaxor	6.0	0.02	1.3	0.03	1.8
QuiltXcel	26.0	0.06	3.0	0.05	3.8
Trivapro	13.7	0.05	2.7	0.02	2.0
	***P*-value**	0.30	0.50	0.17	0.23

[1] NA: Not applicable.