WASHINGTON TURFGRASS SEED COMMISSION
PROGRESS REPORT FOR 2021 PROJECTS

Project No.:_____

Title: Integrated Disease Management of Ergot in Kentucky Bluegrass

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Cooperators: Jory Iverson, David Prior

Reporting Period: November 2020-November 2021

Accomplishments:
The Ergot Alert Network is limited in the scope and number of locations that can be monitored due to equipment costs, labor, and time restrictions. We hypothesized that a citizen science approach can overcome these logistical and technical limitations and empower growers to conduct spore trapping and detection in their own fields. Towards this end, we deployed rotating-arm spore traps to volunteer growers (4 farms) in an effort to expand the Ergot Alert Network and enlist grass seed stakeholders in participatory research and disease monitoring. We also developed and validated a field-deployable DNA extraction protocol and recombinase polymerase amplification (RPA) assay, coupled with a lateral flow dipstick (LFD), for the sensitive and visual detection of *Claviceps* spores in spore traps by growers. The RPA-LFD assay was sensitive to 100 spores/sample (or 2 spores/reaction) and could detect both *C. purpurea* and *C. humidiphila*, the two causal agents of ergot in PNW grass seed crops. The ability to distribute inexpensive spore traps to growers upon request will allow researchers to increase the scope of the Ergot Alert Network and provide spore counts to more growers across the Pacific Northwest. The RPA assay will allow growers to perform a rapid, visual and inexpensive (about $10/test) DNA-based test to detect ergot spores during the growing season. Risk-based fungicide applications based on the presence of inoculum should contribute to more targeted and effective fungicide applications by growers in Washington.

Results:
Objective 1: A spore trap network was established with grass seed cooperators in Washington (1 farm) and Oregon (3 farms) who volunteered for the program. Sample collection rods were changed weekly. Samples were processed using qPCR and the results were provided directly to the cooperator and on the Ergot Alert Blog.

Spore trapping for the 2021 Ergot Alert Network was started on April 29 at most locations. Burkard spore traps were located at the Hermiston Agricultural Research and Extension Center, the Central Oregon Agricultural Research and Extension Center, and in a commercial field near Paterson, WA. Rotating arm samplers were also placed in a second commercial field near Paterson, WA, two commercial fields in Union County, OR, and three commercial fields near Echo, OR. Depending on the location, spore sampling was halted between June 15 and June 23. A total of five ergot alerts were released to growers during the season via the COAREC Ergot Alert Blog.

Objective 2: Three DNA extraction methods (NaOH-based, Instagene, and boil-lysis) were tested using spore suspensions of 1, 10, 100, and 1,000 spores/ml. DNA quantity and quality were measured using a fluorometer and spectrophotometer, respectively. A crude boil-lysis DNA extraction protocol (8 min. of boiling in 0.1 M Tris buffer) was selected to extract DNA from spore suspensions containing 1, 10, 1,000, and 10,000 spores.

RPA primers were designed based on sequence polymorphisms of either the internal transcribed spacer (ITS) region or beta-tubulin gene of *Claviceps purpurea*. ITS and beta-tubulin sequences of *Claviceps* species available at the National Center for Biotechnology Information (NCBI) were used for primer design. Six RPA primers were designed and screened for specificity and sensitivity through conventional PCR. Primers targeting the beta-tubulin gene were selected and tested against 23 *C. purpurea* isolates,13 *C. humidiphila* isolates, 6 other *Claviceps* species, 22 rotating-arm samples collected from the field, and 2 non-target fungal species (*Verticillium dahliae* and *Fusarium oxysporum*).

In order for the RPA products to be visualized by a lateral flow device (LFD), a specific internal probe was designed based on the sequence of the selected primer pair. The RPA products were mixed with the dipstick assay buffer, transferred to the sample pad of the strip, and running buffer added prior to incubation. DNA amplification was observed with the naked eye as indicated by the test line. The RPA-LFD assay was sensitive to 100 spores/sample, or 2 spores/RPA reaction of both *C. purpurea* (Fig.1) and *C. humidiphila*. (data not shown)**.**

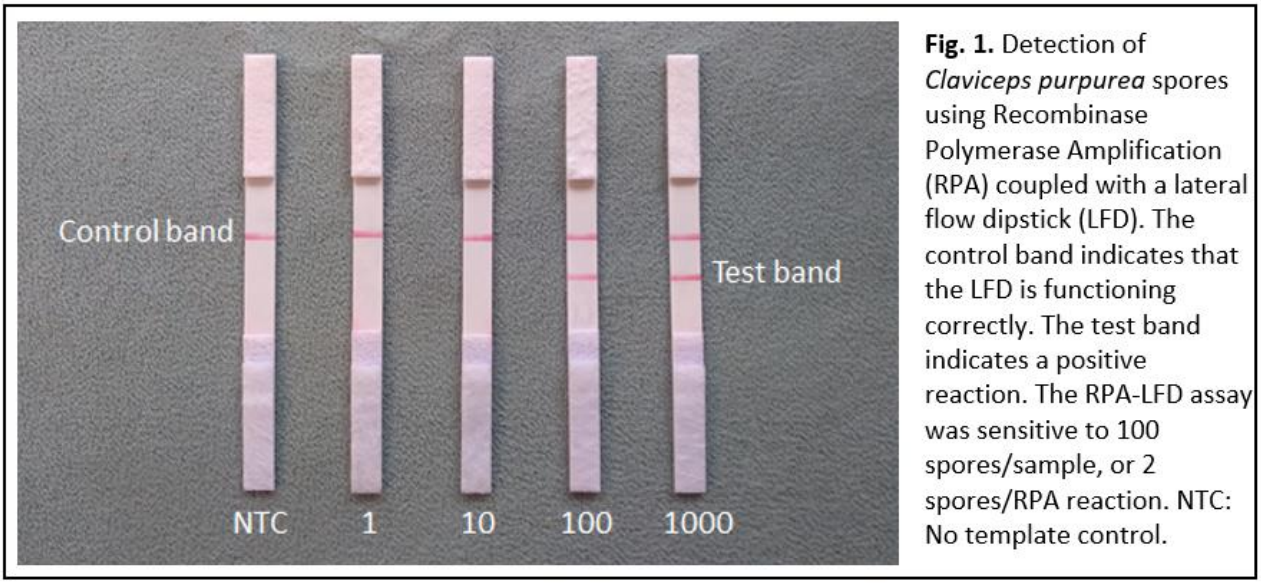

Fig. 1. Detection of *Claviceps purpurea* spores using Recombinase Polymerase Amplification (RPA) coupled with a lateral flow dipstick (LFD). The control band indicates that the LFD is functioning correctly. The test band indicates a positive reaction. The RPA-LFD assay was sensitive to 100 spores/sample, or 2 spores/RPA reaction. NTC: No template control.

Publications:

Dung, J.K.S., Duringer, J.M., Kaur, N., Scott, J.C., Frost, K., Walenta, D.L., Alderman, S., Craig, A.M., and Hamm, P. 2021. Molecular and alkaloid characterization of *Claviceps purpurea* sensu lato from grass seed production areas of the U.S. Pacific Northwest. Phytopathology 111(5):831-841 doi.org/10.1094/PHYTO-07-20-0289-R

Cheng, Q., Frost, K.E., and Dung, J.K.S.. 2020. Population genetic structure of *Claviceps purpurea* in cool-season grass seed crops of Oregon. Phytopathology 110(11):1173-1780. doi.org/10.1094/PHYTO-01-20-0005-R

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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 Garlic and Onion Research Advisory Board	$7,700	March 2020 – February 2022	1%	Genetic diversity and pathogenicity of *Fusarium proliferatum* causing clove rot on garlic
Dung, Frost	Oregon Seed Council	$18,293	July 2021 - June 2022	1%	Enhanced IPM of Ergot in Grass Seed Crops
Dung, Frost, Cheng, Walenta,	Washington Turfgrass Seed Commission	$26,567	July 2021- June 2022	2%	Integrated Disease Management of Ergot in Kentucky Bluegrass
Dung, Chang, Mahmud, Mahaffee, Jacobs, Greenway, Savory, du Toit, Sidhu, Stoll	USDA-NIFA SCRI	$2,999,364 (Dung: $655,067)	September 2020-August 2024	10%	A Systems Approach for Managing Bacterial Blight of Carrot

Dung	Mint Industry Research Council	$89,050	July 2019 – June 2022	2%	Identifying Economic Action Thresholds to Inform Verticillium Wilt Management Decisions
H. Pappu, J. Dung, C. Cramer, B. Nault, M. Havey	USDA-NIFA-SCRI	$3,291,766 (Dung: $449,946)	September 2018 – September 2022	5%	Managing Stakeholder-Prioritized Pests and Diseases Threatening the US *Allium* Industry
Dung, Frost	Eastern Oregon Kentucky Bluegrass Workgroup	$18,293	July 2021-June 2022	1%	Integrated Disease Management of Ergot in Kentucky Bluegrass
Dung, Spring, Sullivan	Eastern Oregon Kentucky Bluegrass Workgroup	$9,394	July 2021-June 2022	1%	Revisiting Plant Growth Regulator Rates Under Different Soil Moisture Conditions
	Pending:				
Dung	Agricultural Research Foundation	$14,993	February 2022– January 2024	1%	Developing Population Biology Resources for the Integrated Management of Fusarium Bulb Rot in Oregon and U.S. Garlic Production
Dung	California Garlic and Onion Research Advisory Board	$22,944	March 2022 - February 2023	1%	Population Biology and Integrated Management of *Fusarium proliferatum* in U.S. Garlic Production
Dung	Mint Industry Research Council	$54,458	July 2022 – June 2023	2%	Integrated Management of Verticillium Wilt in Mint