AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE: Development of a field-deployable assay for detecting ergot spores in grass seed production systems

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SUMMARY/ABSTRACT:

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 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* 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. A fungicide timing trial was also conducted in artificially-infested perennial ryegrass (cv. 'Pavilion') plots established at the Hermiston Agricultural Research and Extension Center. Fungicides were applied at late boot-early heading, anthesis, or both timings and compared to non-treated plots. All timings significantly reduced ergot compared to the non-treated control, but differences between fungicide timings were not observed.

OBJECTIVES:

The purpose of this proposal was to develop a rapid, visual and inexpensive molecular assay (RPA-LFD assay) for the detection of airborne *C. purpurea* spores. The assay could ultimately be used by grass seed growers to make decisions regarding fungicide applications for ergot management based on the presence of absence of inoculum. Objective 1 was to develop and validate a crude field-deployable DNA extraction protocol. Objective 2 was to develop an RPA assay, coupled with a LFD, which is suitable for the sensitive and specific detection of *C. purpurea* in the field.

PROCEDURES:

Objective 1. Three DNA extraction methods (NaOH-based, Instagene, and crude 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.

The RPA primers and probe were designed to target the beta-tubulin gene of *C. purpurea* according to RPA guidelines and manufacturer's instructions for the Agdia AmplifyRP® Discovery Kit. To determine the optimal conditions for the RPA assay, different combinations of reaction temperatures and reaction times were also evaluated. Primers were tested against 42 *Claviceps* isolates and 22 spore sampling rods.

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.

SIGNIFICANT ACCOMPLISHMENTS:

A crude boil-lysis DNA extraction protocol (8 min. of boiling in 0.1 M Tris buffer) was selected to extract DNA based on assay simplicity, reagent availability, and DNA quantity and quality. DNA was extracted using this method 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. The primers were 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*).

The crude DNA extract was then subjected to RPA and the samples were loaded onto LFD. The RPA-LFD assay was sensitive to 100 spores/sample, or 2 spores/RPA reaction of both *C. purpurea* (Fig.1) and *C. humidiphila*, another *Claviceps* species that can cause ergot in grass seed crops (data not shown)

BENEFITS & IMPACT:

The ability to distribute inexpensive spore traps to growers and field-deployable assays 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 or absence of inoculum should contribute to more targeted and effective fungicide applications by growers in Oregon.

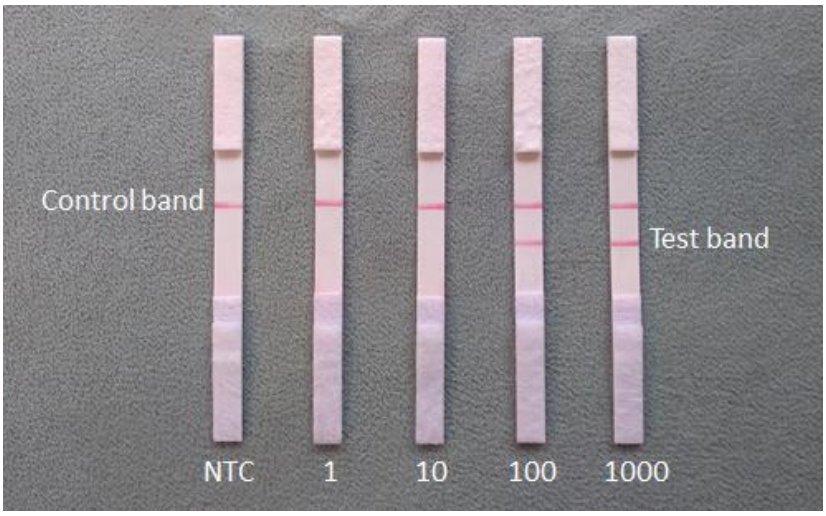

Fig. 1. Detection of *Claviceps purpurea* spores using Recombinase Polymerase Amplification (RPA) coupled with a lateral flow dipstick (LFD). The control band indicates that the LFD is functioning correctly. The test band indicates a positive reaction. The RPA-LFD assay was sensitive to 100 spores/sample,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

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

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FUTURE FUNDING POSSIBILITIES:

None identified to date.