DEVELOPING ERGOT DISEASE DETECTION TECHNOLOGY FOR ENHANCED IPM IN GRASS SEED CROPS

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Introduction

Ergot is an important seed replacement disease of perennial ryegrass and Kentucky bluegrass (KBG) seed crops grown in irrigated production regions of the lower Columbia Basin of Washington and Oregon. The ergot fungus (*Claviceps purpurea*) infects unfertilized flowers of grasses and transforms ovaries into dormant resting structures (sclerotia), which overwinter and produce spores the following season. The disease reduces yield and can be difficult to manage with fungicides alone (Cheng et al., 2019). Additional losses are incurred during seed cleaning to remove ergot.

Since 2015, our research team has provided regional Ergot Alerts to stakeholders during the grass seed production season (Walenta et al., 2016). The weekly Ergot Alerts provide growers and crop consultants in the Columbia Basin, Grande Ronde Valley, and central Oregon with disease risk potential based on spore counts and a predictive model. Recently, ergot has become a disease of concern in irrigated production regions of the upper Columbia Basin. The disease has also been reported in dryland production regions. As reports of ergot expand across the Pacific Northwest, a need exists for expanding disease monitoring and modeling efforts. However, the number and geographic range of Ergot Alert sites that can be monitored are limited due to the cost of equipment and the time required to travel to each site for sample collection.

Materials and Methods

Instead of Burkard spore samplers, which are relatively expensive (more than $5,000) to purchase and maintain, we deployed rotating-arm samplers, which were relatively inexpensive (less than $100) and easy to build and maintain. Validation of the rotating-arm samplers was performed at the Central Oregon Agricultural Research and Extension Center (COAREC, Madras, OR) in a 5-acre, second-year KBG seed field with a history of ergot. Three plots were established in the 5-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. The three rotating-arm samplers in each plot were set at different heights (2, 3, and 4 feet) to test the effect of sampling height on spore collection efficiency. Different sampling periods (1, 2, 3, 4, and 7 days) were 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

During the first year of validation, 238 sampling events were performed, and ergot spores were detected on 211 (88.7%) of the sampling days. Detection events 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 greatest in the 4-foot rotating-arm sampler and Burkard spore sampler (seven false negatives each), followed by the 2-foot rotating-arm sampler (four false negatives) and the 3-foot rotating-arm sampler (two false negatives) (Table 1).

Results from rotating-arm samplers set at collection heights of 2, 3, and 4 feet were compared with those from the standard Burkard spore sampler, which collects at a height of 2 feet. There were no statistical differences among collection heights and the Burkard sampler ($P = 0.15$) (Table 2), indicating that rotating-arm samplers performed equally at different collection heights and were comparable to the standard Burkard spore sampler. As the season progressed, operation of the rotating-arm samplers at 2-foot heights was compromised by the canopy, so it will be recommended that rotating-arm samplers be placed just above the expected canopy height at anthesis.

Different sampling periods (1, 2, 3, 4, and 7 days) were tested and compared with the standard Burkard spore sampler. There was a significant difference among sampling periods ($P < 0.0001$) (Table 3). Overall,

Table 1. 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 (collects at a height of 2 feet).

Sampling period	Sampling height	Detected	Not detected	False negatives[1]
1 day	Rotating-arm (2 ft)	2	1	0
	Rotating-arm (3 ft)	2	1	0
	Rotating-arm (4 ft)	0	0	2
	Burkard (2 ft)	1	1	1
2 days	Rotating-arm (2 ft)	6	0	0
	Rotating-arm (3 ft)	6	0	0
	Rotating-arm (4 ft)	6	0	0
	Burkard (2 ft)	6	0	0
3 days	Rotating-arm (2 ft)	26	1	3
	Rotating-arm (3 ft)	28	1	1
	Rotating-arm (4 ft)	25	1	3
	Burkard (2 ft)	23	1	6
4 days	Rotating-arm (2 ft)	14	0	1
	Rotating-arm (3 ft)	14	0	1
	Rotating-arm (4 ft)	13	0	2
	Burkard (2 ft)	15	0	0
7 days	Rotating-arm (2 ft)	6	0	0
	Rotating-arm (3 ft)	6	0	0
	Rotating-arm (4 ft)	6	0	0
	Burkard (2 ft)	6	0	0

[1]False negatives are 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.

ergot detection was greatest in samples collected every 7 days, but the 2-day sampling period was not different from the 7-day sampling period (Table 3).

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 ($P = 0.32$) (data not shown). Samples collected for 7 days performed best during precipitation events but were not different than samples collected after 2 days (data not shown); these results corresponded to those from samples collected under all weather conditions.

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

Sampling height	Mean cycle threshold value[1]
Rotating-arm (4 ft) ($n = 58$)	30.33
Rotating-arm (3 ft) ($n = 60$)	28.24
Rotating-arm (2 ft) ($n = 60$)	28.23
Burkard (2 ft) ($n = 60$)	28.16
P-value	0.15 NS[2]

[1]A smaller cycle threshold value indicates that more spores were collected.
[2]NS = not significant at 0.05 level.

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

Sampling period	Mean cycle threshold value[1]
(number of samples)	
1 day (n = 11)	35.25 a
3 day (n = 119)	29.28 b
4 day (n = 60)	28.16 bc
2 day (n = 24)	25.73 cd
7 day (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 by Tukey's test.

Conclusion

Overall, 91.6% of the 238 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 the rotating-arm samplers should be placed above the crop canopy. A minimum sampling period of at least 2 days would be 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 rotating-arm spore samplers can perform as well as standard Burkard spore samplers for monitoring airborne ergot ascospores. These results will be further validated in the second year.

References

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