Timing of Occurrence of *Claviceps purpurea* Ascospores in Northeast Oregon

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**Abstract**

Ergot, caused by *Claviceps purpurea*, is an important floral disease of grasses, characterized by sclerotium formation within the host flowers. To determine whether annual variation in ergot severity in Kentucky bluegrass is a result of ascospore density and/or timing of ascospore occurrence, Burkard 7-day volumetric spores traps were used to monitor ascospores of *C. purpurea* in each of two Kentucky bluegrass fields in the Grand Ronde Valley in northeastern Oregon between mid-May and late June, 2008-2010. Ascospores were typically trapped between midnight and 6:00 a.m. In 2008 and 2010, most ascospores were released prior to flowering in Kentucky bluegrass, corresponding to no observed ergot in 2008 and a low level of ergot in 2010. In 2009, ascospore release and pollination coincided, but few airborne ascospores were present, resulting in a low level of ergot. Similar ergot levels were observed in fungicide trials, suggesting that fungicides for ergot control were unnecessary. In years when there are few ascospores during flowering in Kentucky bluegrass, a reduction of up to two fungicide applications may be possible.

**Introduction**

In Union County in northeastern Oregon, Kentucky bluegrass (*Poa pratensis* L.) is grown for seed on about 2511 ha, with nearly all of the fields located in the Grand Ronde Valley. In 2009, 2.1 million kg of Kentucky bluegrass seed were produced, with a farm gate value of $4.8 million (16).

Ergot, caused by *Claviceps purpurea* (Fr:Fr.) Tul., is a persistent problem in Kentucky bluegrass in northeastern Oregon, with direct seed loss as great as 25% (3). Seed loss can occur directly from replacement of seed with sclerotia, or indirectly when the sugary, sticky “honeydew” stage, a mixture of conidia and plant sap, which precedes formation of sclerotia, clumps seeds and debris together, and sticks to machinery during harvest (11). Additional seed loss occurs when recleaning is required to remove ergot to meet certification standards (3).

Ascospores, the primary inoculum, produced from overwintered sclerotia during the spring about the time of flowering in grasses (1,4,14), infect the grass ovaries. Honeydew, visible within one to two weeks after infection (7,13,17), attracts insects, especially flies and moths (5,9), which are presumed responsible for secondary spread of conidia. Infected ovaries are replaced with elongated black sclerotia, also referred to as ergot. Sclerotia fall to the ground, overwinter, and germinate in the spring (14,18) to produce stalked, spherical stromata embedded with perithecia. Ascospores are forcibly discharged and dispersed by wind.

Ergot severity can vary from year to year. Understanding the nature of the variability is important in developing control strategies for ergot. The objectives of this study were to: (i) determine whether variation in the severity of ergot in Kentucky bluegrass is related to the timing and/or number of airborne ascospores during flowering; (ii) determine when the unfertilized grass ovaries...
are susceptible to infection; and (iii) evaluate the efficacy of timing fungicide sprays for ergot control.

Field Sites for Ascospore Trapping and Fungicide Trials

In 2008, two established ergot-infested commercial Kentucky bluegrass fields in the Grand Ronde Valley in northeastern Oregon were selected for ascospore trapping. Site 1 was a 16.2-ha field, cv. Midnight II, planted in May 2004 and irrigated with a wheel-line sprinkler. Site 2 was a 28.4-ha field, cv. SR 2100, planted in May 2004 and watered with a center pivot irrigation system. Site 2 was about 3.2 km southeast of Site 1. In 2009 Site 1 was taken out of production and replaced by Site 3, a similar field (27 ha, cv. Midnight II planted May 2004) and located about 0.8 km northwest of Site 2.

A fungicide trial was established at Site 1 in 2008 and Site 3 in 2009 and 2010 to determine the efficacy of both timing and number of fungicide applications needed to control ergot. Quilt was applied at the label rate (168 ml/ha) and included 1% v/v stylet oil. Treatments in 2008 included: (i) a single application at early heading; an application at early heading and early flowering; (ii) an application at early heading, early flowering, and post flowering; and (iii) untreated control. Plot size was set at 27 m by 305 m to accommodate a commercial field applicator with 27-m boom, a swather, and a combine. Sprayer volume was 150 liter/ha at early heading and 168 liter/ha at early and post flowering, at 30 psi. The experimental design was a randomized block with 3 replications.

In 2009 and 2010 a single application at early flowering and a single application 14 days later (post flowering) were included among the treatments. Plot design in 2009 and 2010 was changed to utilize small plot equipment. Each plot was 1.5 m × 9.1 m. Fungicide treatments were applied with a hand-held CO2 sprayer with 1.5-m hand boom fitted with TeeFet TurboJet 60-11003 nozzle tips, delivering a spray volume of 168 liter/ha at 30 psi. The experimental design was a randomized block with 4 replications.

In all years and sites, fertilization, weed/insect management, and irrigation were managed by the cooperating grower and followed common production practices for the area. In 2008, 2009, and 2010, plots were swathed on July 12, 15, and 26, and harvested on August 1, July 31, and August 5, respectively. In 2008, two swaths were harvested per plot, with harvested area in each plot 8.8 m by 182 m. A weigh wagon was used to measure bulk seed weight. A 3.8-liter can was used to sample seed as it was augered from the combine to weight wagon. Five samples were taken, timed to collect a representative sample from the entire seed volume, and bulked into a 19-liter bucket. In 2009 and 2010 the entire 1.5-m × 9.1-m plot was harvested with a Hege plot combine to determine bulk seed yield. Samples collected during harvest were cleaned and seed yield and percentage ergot contamination (w/w) was determined. Grass seed yield and percentage ergot contamination were subject to ANOVA and means were separated by the LSD all pair-wise comparison test. Data were analyzed using Statistix 9 (Analytical Software, Tallahassee FL).

Fungicide Trial

Ergot was not detected in the fungicide trial plots in 2008. A low level of ergot (< 0.51%) occurred in 2009 and 2010 (Table 1), but there was no significant difference in the level of ergot among the treatments.
Table 1. Fungicide treatment, application date, seed yield, and percentage ergot at Site 1 or 3 during 2008, 2009, and 2010.

<table>
<thead>
<tr>
<th>Fungicide application</th>
<th>Date</th>
<th>Clean seed yield (kg/ha)</th>
<th>% ergot*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2008 (Site 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>early heading</td>
<td>May 28</td>
<td>1549</td>
<td>0</td>
</tr>
<tr>
<td>early heading + early flowering</td>
<td>June 17</td>
<td>1622</td>
<td>0</td>
</tr>
<tr>
<td>early heading + early flowering + post flowering</td>
<td>June 27</td>
<td>1542</td>
<td>0</td>
</tr>
<tr>
<td>untreated</td>
<td></td>
<td>1533</td>
<td>0</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>244 ns</td>
<td>0 ns</td>
</tr>
<tr>
<td>2009 (Site 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>early heading</td>
<td>May 29</td>
<td>935</td>
<td>0.31</td>
</tr>
<tr>
<td>early flowering</td>
<td>June 8</td>
<td>963</td>
<td>0.15</td>
</tr>
<tr>
<td>post flowering</td>
<td>June 23</td>
<td>1005</td>
<td>0.34</td>
</tr>
<tr>
<td>early heading + early flowering</td>
<td></td>
<td>1005</td>
<td>0.21</td>
</tr>
<tr>
<td>early heading + early flowering + post flowering</td>
<td></td>
<td>960</td>
<td>0.13</td>
</tr>
<tr>
<td>untreated</td>
<td></td>
<td>976</td>
<td>0.43</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>121 ns</td>
<td>0.51 ns</td>
</tr>
<tr>
<td>2010 (Site 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>early heading</td>
<td>June 1</td>
<td>796</td>
<td>0.11</td>
</tr>
<tr>
<td>early flowering</td>
<td>June 14</td>
<td>708</td>
<td>0.04</td>
</tr>
<tr>
<td>post flowering</td>
<td>July 1</td>
<td>557</td>
<td>0.09</td>
</tr>
<tr>
<td>early heading + early flowering</td>
<td></td>
<td>683</td>
<td>0.03</td>
</tr>
<tr>
<td>early heading + early flowering + post flowering</td>
<td></td>
<td>651</td>
<td>0.01</td>
</tr>
<tr>
<td>untreated</td>
<td></td>
<td>679</td>
<td>0.03</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td></td>
<td>255 ns</td>
<td>0.07 ns</td>
</tr>
</tbody>
</table>

* Percentage ergot based on w/w ergot/clean seed.
ns = not significant.

**Spore and Pollen Trapping**

Ascospores were monitored using Burkard 7-day volumetric spore traps (Burkard, Rickmansworth, England). The traps were placed within fields and spore tapes were prepared, processed, and examined as previously described (1). At each site, the spore trap orifice was approximately 42 cm above the ground and air intake was adjusted to 10 liters/min. Air intake was checked at least once weekly. The number of *C. purpurea* ascospores and grass pollen were counted under a microscope at 100 to 400× and summed over 12:00 a.m. to 11:59 p.m. each day to establish daily counts. Standard slides of *C. purpurea* ascospores were compared to known standards.

The duration of flowering and pollen production were determined by pollen counts of the Burkard spore trap tapes. Pollen were compared with known standards of Kentucky bluegrass pollen. Differentiation of pollen to grass species was not attempted.

**Periodicity of Ascospore and Pollen Release**

Most ascospores of *C. purpurea* were trapped between 1:00 and 8:00 a.m. (Fig. 1) with few to no spores trapped between 12:00 p.m. to 5:00 p.m. The occurrence of ascospores during the late evening through early morning hours is consistent with previously published data (1).
Fig. 1. Percentage of total ascospores trapped at each hour of the day at Sites 1 or 3 and Site 2, 2008, 2009, and 2010.

Fewest grass pollen were trapped between 5:00 and 6:00 a.m., followed by small peak between about 8:00 and 11:00 a.m. (Fig. 2). The scarcity or lack of spores during the afternoon suggests that in the Grand Ronde Valley, grass species that flower in the afternoon would be at less risk than Kentucky bluegrass, which typically flowers at night or morning, depending on cultivar (12). Resistance to ergot infection follows host fertilization (8), and consequently, flowers that escape infection and are pollinated later in the morning would develop resistance to infection.
Timing of Ascospore and Pollen Release
Ascospores were trapped from mid-May through mid- to late June (Fig. 3). Total ascospores trapped at Site 1 in 2008, 2009, and 2010 were 7599, 181, and 1137, respectively. At Site 2 in 2008, 2009, and 2010 total ascospores trapped were 1332, 106, and 734, respectively. The long period of spore release is consistent with previous ergot spore trapping studies (1,4,14), and is likely a reflection of variation in the timing of germination of sclerotia which can extend for weeks (14,15).
The pattern of ascospore release was similar and ascospore numbers among the three sites were more or less parallel, suggesting that environmental conditions were similar among sites. The appearance of ascospores as early as May 14 in 2009 and 2010 (when spore trapping started) was several weeks in advance of flowering in Kentucky bluegrass. The early appearance of ascospores suggests that early flowering grasses such as annual bluegrass (Poa annua L.), which can flower weeks earlier than Kentucky bluegrass could be at risk of infection and potentially provide a source of secondary inoculum. In addition, weed grasses such as cheatgrass (Bromus tectorum L.) and witchgrass (Panicum capillare L.) are common in Kentucky bluegrass fields in the Grand Ronde Valley and could potentially contribute sclerotia to the field, increasing the disease potential the following year.

In 2009, grass pollen was trapped about a week earlier than in 2008 and 2010 (Fig. 4).
Similarly, observed flowering in Kentucky bluegrass was earlier in 2009 and corresponded to a faster accumulation of degree days (Fig. 5) relative to 2008 and 2010.
Most of the ascospores were released before the start of flowering, resulting in the crop escaping infection. In 2009, a greater overlap between ascospores and pollen occurred, although few ascospores were present, and at numbers that were likely to promote only a low level of infection. Warm spring temperatures, which promote early flowering in Kentucky bluegrass, could place the crop at greater risk for infection if there is greater overlap with ascospore occurrence.

It is not clear if the ascospores trapped at each site originated solely from within the fields included in the study, or from other fields or areas. In an area such as the Grand Ronde Valley, where winds are common and wind direction variable, a single infested field could be a significant source of inoculum for surrounding fields.

Susceptibility to ergot varies among cultivars, and ranges from highly susceptible to resistant (2). In highly susceptible cultivars, fungicides alone would likely provide inadequate control. Attempts to control ergot with fungicides have been met with mix results (6,10).

It is not clear to what extent secondary spread of ergot occurred in the plots as honeydew occurrence was not quantified. The potential for secondary spread depends on the period that seed heads emerge and the duration of flowering within seed heads. Cultivars in which most seed heads emerge about the same time and in which flowering duration is short will have the least risk for secondary spread, as there will be few newly opened and unfertilized flowers available for infection by the time that honeydew is produced. In cultivars with a longer flowering duration, secondary spread would depend on the number and movement of insects capable of transferring conidia from infected to uninfected, unfertilized flowers. It remains to be determined if irrigation washes honeydew off plants or contributes to secondary spread though rain splash.

**Implications for Control**

From an ergot management perspective, cultivars highly susceptible to ergot should be avoided when possible, alternate hosts for *C. purpurea* should be strictly controlled both within and surrounding the field, and efficacious fungicides should be applied at the beginning of flowering. In addition, it may be possible to reduce the number of sclerotia in the field following harvest by post-harvest field burning. Currently, growers typically spray for ergot early to mid-June, depending on start of flowering, with a second application 14 days later. Results from this study suggest that monitoring the level of airborne ascospores prior to and during flowering in Kentucky bluegrass might provide a means to determine whether or not to apply up to two fungicide sprays for ergot control. On a broader level, a degree day model to predict early flowering in Kentucky bluegrass may have potential to predict years in which the crop may be at greater risk for ergot. Additional studies will be needed to establish the potential of these approaches as decision aides to eliminate one or both fungicide sprays.
Acknowledgments and Disclaimers

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Literature Cited