Environmental Factors Associated with Ergot Ascospore Production in Seed in Central Oregon and Evaluation of Ergot Resistance/Klendusity in Kentucky Bluegrass Cultivars

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Introduction

Ergot is an important disease of Kentucky bluegrass and can be a persistent problem in seed production systems. The disease is caused by the fungal pathogen *Claviceps purpurea*, which has a very wide host range among grasses and grains in North America. The fungus infects flowers prior to fertilization and colonizes the ovaries, resulting in the production of sclerotia rather than viable seed. Sclerotia are the overwintering structures of the fungus and produce airborne ascospores that serve as primary inoculum the following growing season. Ergot infection and infestation incurs economic losses at various stages of grass seed production, including direct yield loss due to the production of sclerotia instead of seed, costs associated with protective fungicide applications, seed loss during re-cleaning processes that are required to remove ergot sclerotia from infested seed lots, and rejection of certification.

Cool-season grass seed is produced in a wide range of climates in Oregon, ranging from mild and moist conditions in the Willamette Valley, semi-arid high elevation deserts in central Oregon and the Columbia Basin of eastern Oregon, and high mountain valleys in northeastern Oregon. Consequently, the incidence and severity of ergot epidemics in grass grown for seed can vary among and within growing regions and from year to year. In some years the timing of ascospore release by the fungus may not coincide with grass flowering (anthesis), which is the only period of host susceptibility. Cultivars with short, uniform flowering periods, or cultivars that flower outside of periods of peak spore production, may potentially escape ergot infection.

Previous studies have investigated the timing and aerobiology of ergot ascospores production in western and eastern Oregon. In the Willamette Valley, ascospore release in the field was associated with rain events occurring 2 to 3 days prior and was not correlated with temperature or relative humidity. In the high desert of central Oregon, grass seed production fields are frequently irrigated and air or soil temperatures may play more important roles in ascospore release than precipitation events. A further understanding of the environmental conditions that contribute to ergot ascospore production in central Oregon grass seed production systems is needed and could provide information that can be used to predict ascospore release and improve the timing of fungicide applications.

The objectives of this study were to: 1) evaluate Kentucky bluegrass cultivars for the potential to escape or resist ergot infection under central Oregon field conditions; and 2) determine the seasonal timing and concentration of ergot ascospores in central Oregon and identify environmental factors that contribute to ascospore production. It was hypothesized that cultivars which flower before or after peak ergot spore production, or those with shortened periods of anthesis, would escape infection more than those which flower when ergot spores are present at high levels.The ultimate goal is to develop a model that can be used to predict ascospore production events in Kentucky bluegrass seed fields located in central Oregon.

Materials and Methods

Evaluation of Kentucky bluegrass cultivars for disease escape potential
A total of 12 Kentucky bluegrass cultivars ('Blue Ghost', 'Gateway', 'Shamrock', 'Bluechip', 'Gladstone', 'Nuglade', 'PST-K4-7', 'Fielder', 'Midnight II', 'Jumpstart', 'Right', and 'DB-1013') were planted in plots at COARC in August 2014. Plots (26 ft long and 5 ft wide consisting of 6 rows of plants) were planted with each cultivar at a seeding rate of 5 lb seed/acre. Each plot was replicated four times and cultivars were arranged in a randomized complete block design. The border of the experiment area was artificially infested in October 2014 with Kentucky bluegrass sclerotia collected from seed lots produced in central Oregon.

Crop phenology was assessed weekly between May 14 and June 10, 2015 to determine the timing and duration of anthesis for each Kentucky bluegrass cultivar. Crop phenology was measured using the Feekes scale, whereby the appearance of stigmas and/or anthers was considered the beginning of flowering (stage 10.51). The percentage of tillers at each Feekes stage were estimated in each plot. Flowering was considered to be completed with at least 90% of the plot reached Feekes stage 11.1. Disease incidence (number of infected seed heads) and severity (number of sclerotia) were determined from a random sample of 100 seed heads collected from each plot at harvest. Data were analyzed using ANOVA and multiple comparisons were made using Tukey's test.

Spore sampling, environmental data collection, and model development
A Burkard 7-day recording volumetric spore sampler was used to collect airborne ascospores of *C. purpurea* in the Kentucky bluegrass plots described above. The spore sampler was placed in the middle of the plots from April 11, 2015 and June 23, 2015 with the air intake orifice located approximately 2 ft above the soil. Spore trap tapes were replaced weekly and each tape was cut into daily segments, stained, and the number of *C. purpurea* ascospores were determined for each hour and then totaled to establish daily counts.

Air and soil temperatures, relative humidity, dew point, and soil moisture were monitored at hourly intervals in each field using a Watchdog 1000 Series Micro Station (Spectrum Technologies, Aurora, IL). Air temperature, relative humidity, and dew point were recorded at a height of 3 ft and soil temperature and moisture were recorded at 2 inches below the soil surface. Similar data, as well as daily precipitation and evapotranspiration, were obtained from the AgriMet MRSO weather station located at COARC. Cumulative air and soil degree days were calculated using air and soil temperature data collected from the MRSO station beginning on January 1. Daily and cumulative degree days were calculated for both air and soil temperatures using a base temperature of 50° F and an upper threshold temperature of 77° F. Base and upper threshold temperatures were based on previous incubation studies which found that ergot sclerotia germination was inhibited by temperatures outside of this range.

Correlation analysis was performed to identify significant correlations between environmental variables and spore counts and logistic regression was used to model daily counts of ascospores with environmental variables. Local regression, which does not assume a linear relationship, and box-and-whisker plots were used to identify trends in daily ascospore counts against significant

environmental variables and visually identify upper and lower threshold values of environmental factors that were significantly associated with ascospore occurrence.

Results and Discussion

Evaluation of KBG cultivars for disease escape potential
Significant differences in anthesis initiation date, anthesis termination date, and anthesis duration were observed among the 12 KBG cultivars ($P \leq 0.003$) (Table 1). Among the cultivars tested, Midnight II exhibited the highest ergot incidence and severity, while Jumpstart and Fielder exhibited the lowest ergot incidence and severity (Table 1). A significant positive correlation was observed between anthesis initiation date and ergot incidence ($P = 0.002$; $r = 0.44$) and severity ($P = 0.01$; $r = 0.37$) was observed, suggesting that cultivars which initiated flowering earlier in the season exhibited reduced ergot in central Oregon. Significant differences in ergot levels were observed among Kentucky bluegrass cultivars. Further research is needed to determine if the differences in ergot levels were due to genetic/physiological resistance to ergot, anthesis periods that did not coincide with peak ergot spore production, or other factors.

Spore sampling, environmental data collection, and model development
Similar to 2014, the first ergot spores were detected on May 20. Although the first occurrence of spores occurred on the same day in 2015 as in 2014, the accumulated air and soil degree days on May 20 were lower in 2015 (air = 185; soil = 143) compared to 2014 (air = 294; soil = 175) suggesting that accumulated air or soil degree days are not a reliable predictor for the first occurrence of ergot spores. A total of 1,087 spores were captured during the 74 day period, and the number of spores captured varied drastically from day-to-day (Fig. 1). Over 42% of the total spores were captured on a single day (June 10), which appeared to be associated with an increase in soil moisture and favorable soil temperatures (between 60 and 70° F) (Fig. 1). Spores were not detected after June 13.

Data for several environmental variables collected from the Watchdog data logger placed in the field and the MRSO weather station at COARC were significantly ($P \leq 0.05$) and positively correlated with ergot spore production (Table 2). Logistic regression using data from the field data logger identified a model that included minimum air temperature, minimum and maximum soil temperatures, dew point, and soil moisture that predicted the presence of ergot spores with over 92% accuracy. Using data from the MRSO weather station, logistic regression identified a model that included minimum and mean air temperature and minimum, maximum and mean soil temperatures that predicted the presence of ergot spores with over 91% accuracy. Local regression and box-and-whisker plots were used to identify upper and lower threshold values for environmental factors significantly correlated with spore production. A minimum air temperature greater than 41° F, minimum soil temperature greater than 50° F, maximum soil temperature less than 71° F, dew point between 40 and 50° F, and a volumetric water content between 18 and 25% in the field were associated with ergot spore production. These results are consistent with controlled studies conducted in incubation chambers which concluded that the highest percentage of ergot sclerotia germination was observed at temperatures between 50 and 77° F and germination was reduced at temperatures below 41°F and above 77° F. Based on the results obtained in this study and in other trials, minimum air temperature, minimum and maximum soil temperatures, dew point, and soil moisture appear to be the important factors contributing to

ergot spore production. Several years of data will be required to develop and test a final model for ergot spore prediction in Central Oregon Kentucky bluegrass seed production.

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Table 1. Anthesis initiation, termination, and duration, total number of ergot spores captured during anthesis, and ergot incidence and severity at harvest for 12 Kentucky bluegrass cultivars grown in artificially infested plots at COARC[1]

Treatment	Anthesis initiation	Anthesis termination	Anthesis duration	Total spores during anthesis	Incidence (%)	Severity
Blue Ghost	136.0 b	153.0 bc	17.0 b	229 c	11 ab	27.5 ab
Gateway	141.0 a	159.0 abc	18.0 b	511 abc	8 ab	11.0 b
Shamrock	134.8 b	153.0 bc	18.3 b	229 c	3 b	11.0 b
Bluechip	134.5 b	161.0 ab	26.5 a	605 b	10 ab	23.0 ab
Gladstone	134.8 b	159.0 abc	24.3 ab	511 abc	11 ab	35.3 ab
Nuglade	143.5 a	164.5 ab	21.0 ab	811 a	10 ab	26.3 ab
PST-K4-7	141.0 a	161.0 ab	20.0 ab	605 ab	5 b	5.0 b
Fielder	135.3 b	155.0 bc	19.8 ab	323 bc	3 b	3.3 b
Midnight II	143.8 a	162.8 ab	19.0 ab	686 ab	17 a	51.8 a
Jumpstart	136.0 b	155.0 bc	19.0 ab	323 bc	2 b	3.8 b
Right	135.3 b	153.0 bc	17.8 b	229 c	4 b	6.0 b
DB-1013	135.8 b	153.0 bc	17.3 b	229 c	7 ab	14.0 b
	$P < 0.0001$	$P < 0.0001$	$P = 0.003$	$P < 0.0001$	$P = 0.0002$	$P = 0.0006$

[1] Means followed by the same letters are not statistically different using Tukey's comparison. Anthesis initiation and termination dates are presented as perpetual Julian days (134 = May 14; 164 = June 12).

Table 2. Correlations (*r*-values) between the number of ergot spores captured and environmental data collected from Watchdog data loggers placed in the field or environmental data collected from the AgriMet MRSO weather station located at COARC[1]

Environmental variable	Field	MRSO
Maximum air temperature	0.38*	0.40*
Minimum air temperature	0.50*	0.52*
Mean air temperature	0.46*	0.47*
Air daily degree days	0.25*	0.46*
Air cumulative degree days	0.33*	0.32*
Maximum soil temperature	-0.02	0.52*
Minimum soil temperature	0.48*	0.47*
Mean soil temperature	0.36*	0.49*
Soil daily degree days	0.30*	0.51*
Soil cumulative degree days	0.35*	0.32*
Relative humidity	0.17	0.04
Dewpoint	0.52*	0.51*
Soil moisture	0.48*	NR[2]
Daily precipitation	NR	-0.11
Evapotranspiration	NR	0.37*

[1] An *r*-value = 1 indicates a perfect correlation, while an *r*-value = 0 indicates no correlation. A * indicates the correlation was significant at $P < 0.05$.

[2] Not recorded

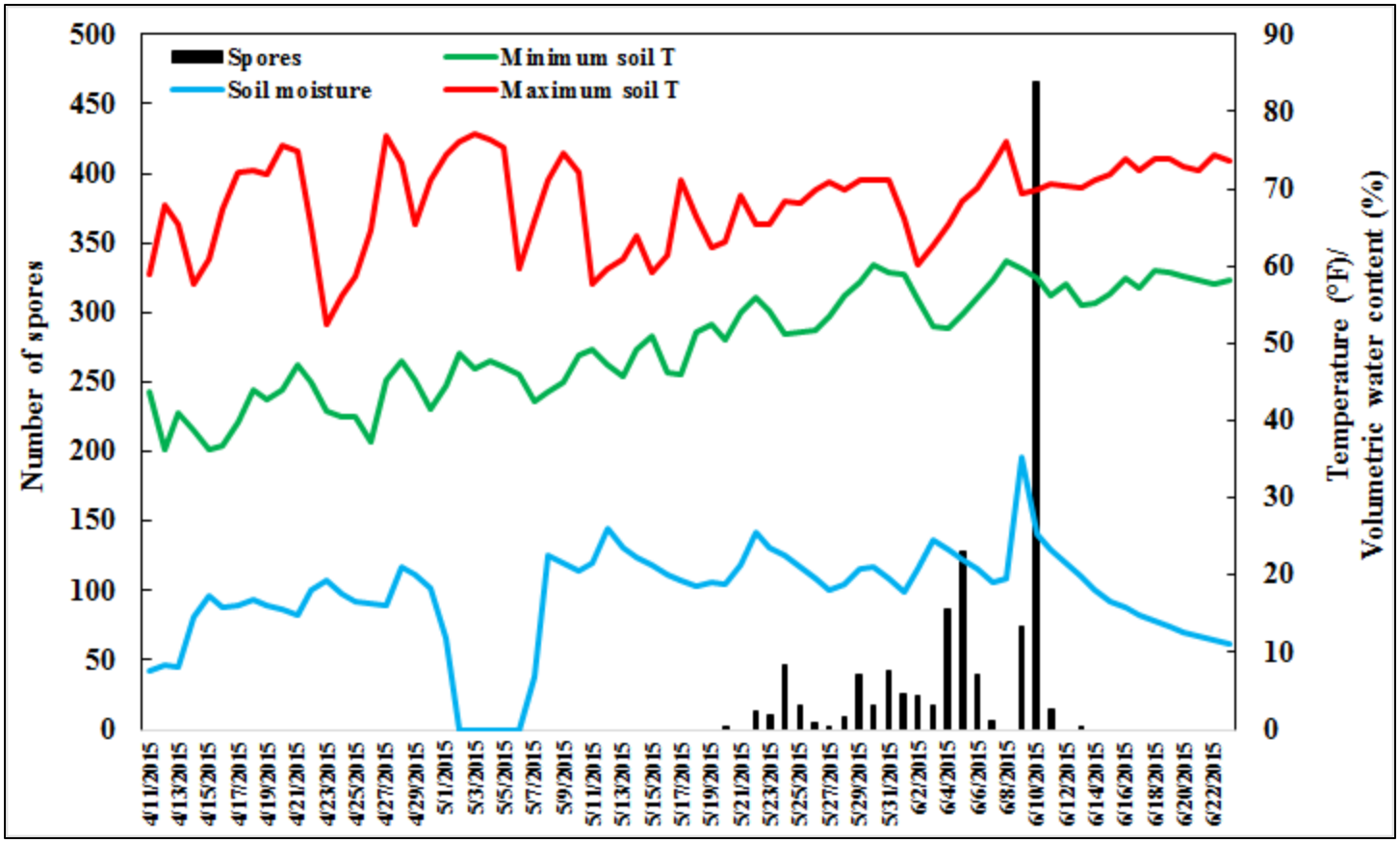

Figure 1. Daily number of ergot spores captured in artificially infested Kentucky bluegrass plots located at COARC (black bars) plotted with minimum daily soil temperature (green line), maximum daily soil temperature (red line), and mean daily soil moisture measured as volumetric water content (blue line).