Studies on Epidemiology of Ergot in Kentucky Bluegrass

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Introduction

Although severity of ergot in Kentucky bluegrass in the Pacific Northwest has varied from year to year, ergot continues to be one of the most economically important diseases in Kentucky bluegrass grown for production seed. At high ergot levels, grass seed becomes contaminated with ergot sclerotia and is rejected or heavily discounted in value. Additional losses are incurred during re-cleaning of seed to remove sclerotia Management of ergot is based on reducing the primary inoculum source through deep ploughing of the field, residue burning, and spraying fungicide to inhibit ascospore production; and by preventing infections through fungicide applications. Reduction of the primary inoculum source has proven to reduce infection, but not to the level to provide adequate control. Deep field ploughing cannot be used in established perennial Kentucky bluegrass; residue burning can only reduce inoculum near the soil surface. Residue burning continues to become less of an option in many parts of the county as it has been progressively banned in many areas due to environmental concerns. The efficacy of spraying fungicides inhibiting germination of ergot bodies in commercial fields has been inadequate, probably due to poor coverage and timing of application. In addition to the crop, susceptible weed grasses within and surrounding the field can also be a source of primary inoculum. Therefore, recent efforts have become more focused on improving the timing of fungicide to protect plants when they are most susceptible to infection, i.e. the flowering period.

Previous studies on release of ascospores of the ergot pathogen, *Claviceps purpurea*, revealed that high ergot severities are associated with high levels of ascospores during the flowering period of grass plants. If ascospores are not available during the flowering period, the only time that the plant is susceptible to infection, ergot will not be a threat and one or more fungicide applications could be saved. In the previous studies in 2009 and 2010, we also found that the ergot sclerotia were aggregated on individual panicles, and that a fungicide applied when ascospores of the pathogen were absent, significantly reduced final disease levels. These suggested that the honeydew, which is essentially plant sap with conidia of the pathogen, may have also contributed to the spread of ergot.

Our objectives in this year were to: 1) quantify the germination of ergot bodies at different temperature conditions with and without preconditioning cold treatment; 2) monitor the release of ascospores and weather conditions in bluegrass fields; 3) compare levels of ergot in plots with and without debris burning; and 4) determine the epidemiological roles of conidia (honeydew) in the spread of the disease in Kentucky bluegrass. Ultimately we hope to construct a predictive model to predict ergot in Kentucky bluegrass and guide fungicide applications.

Materials and Methods

Quantify the Germination of Ergot Bodies at Different Temperature Conditions With and Without a Duration of Cold Treatment

Sclerotia (ergot bodies) of C. purpurea were collected from infected Kentucky bluegrass seeds harvested in 2011 at COARC. Intact ergot bodies were selected, surface sterilized with 2% bleach for 2 minutes, and rinsed three times with sterilized distilled water. Fifty sclerotia were then placed on a polyurethane disc in a foam cup (Figure 1), subject to a 0, 2, 4, 6, and 8- week cold and moist preconditioning period in refrigerator 39.2^{o}F, and followed by incubation at 39.2, 59, 68, 77^{o}F, and 50/68^{o}F 12/12h day/night cycle. The sclerotia were watered twice a week by spraying sterilized distilled water using a hand sprayer. Germination of sclerotia was monitored and recorded twice a week after the first appearance of stromata. Any ergot bodies with discernible stromata were considered germinated. Two cups of sclerotia were included for each treatment. An average germination rate was calculated for each treatment and plotted against incubation time, and days after a cold treatment period.

Monitor the Release of Ascospores and Weather Conditions in Bluegrass Fields in Central Oregon

Weather conditions including air temperature, relative air humidity, soil temperature, soil moisture, precipitation, and solar radiation were collected using a CR3000 Micrologger® (Campbell Scientific Inc., Logan, Utah, USA) in two Kentucky bluegrass fields in central Oregon. The weather data were collected once every 5 minutes and hourly summaries were also recorded. The timing and duration of flowering were determined by visual inspection in the fields. Ascospores and pollen were monitored using a 7-day volumetric spore trap (Burkard Scientific Equipment, UK). The ascospores and grass pollen were pulled into the trap from a vacuum at the base of the trap. Airborne ascospores and pollen were collected on a sticky tape, mounted on a drum with a 7-day rotation cycle with the tape replaced every 7 days. The spores and pollen on the tape were counted and summed up for each day according to their positions on the tape. At harvest, eight 200-head samples were collected and heads with ergot bodies were enumerated.

Compare Levels of Ergot in Plots With and Without Debris Burning

In the established bluegrass variety plots at COARC (established in 2007 by Richard Affeldt to screen varieties for growing without burning treatment, consisting of 4 blocks each), each variety plot was divided into two halves. Debris burning and no burning treatments were then carried out for the two halves in the fall of 2010. In order to create uniform and high disease pressure, 350g of freshly collected sclerotia were distributed into each of the 4 plots with cultivar Volt prior to debris burning treatment. At harvest time in 2011, 200 heads were sampled from each of the halves in plots with varieties: Volt, Atlantis, Merit and A00-891. Ergot bodies on each of these panicles were enumerated and recorded.

Determine the Epidemiological Roles of Conidia (honeydew) in the Spread of the Disease in Kentucky Bluegrass

In each 6 ft × 6 ft plot, five rows of grass plants were transplanted prior to flowering on May 26 in a way to prevent emergence of stromata in the new plots after transplantation. In the absence of ascospores (no sclerotia), the disease was introduced by artificially inoculating early flowers in the center row by spraying twice with a suspension of conidia (10^5 conidia/ml) on June 13 and June 20, and paper boards were used to block the drifts to other rows during the inoculation. The conidia were produced on acid PDA at room temperature in the laboratory. The number of infected heads and number of ergot bodies were enumerated for each row at harvest.

Results and Discussion

Germination of Ergot Bodies at Different Temperature Conditions With and Without a Duration of Cold Treatment

Over all, no germination of ergot bodies was observed when they were incubated at a continuous 39.2^{o}F in this study (data not shown). Unlike results reported previously (Mitchell and Cooke, 1968), a considerable percentage of ergot bodies germinated without a cold treatment when they were incubated at constant 59 or 68^{o}F, or 50/68^{o}F night/day cycles, but not at constant 77^{o}F in this study (Figure 2). However, the time from initiation of incubation to the appearance of the first stromata for treatments incubated at 59, 68, or 50/68^{o}F was longer without cold conditioning than the same treatments with a cold preconditioning treatment (Figure 2). As the length of cold treatment duration increased, the shortest length of this period (among all incubation temperature treatments) decreased from 44 days without cold treatment, to 23, 16, and 13 days for 2, 4 and 6 weeks of cold treatment, respectively, and then increased again to 21 days for 8 weeks of cold treatment (Figure 2). It was also very interesting that more ergot bodies germinated at 77^{o}F as the duration of preconditioning cold treatment increased (Figure 2). The results from this study suggested that preconditioning cold treatment is required only in areas where the temperature is high (at least higher than 68^{o}F). Preconditioning cold treatment can also improve germination (shorter incubation time and higher maximum germination rate) in areas where the soil temperature was 68^{o}F or lower. Regardless of the incubation temperature, the optimal length of preconditioning cold treatment is 4-6 weeks, and prolonged preconditioning cold treatment delays the germination of ergot bodies (Figure 2). The optimal incubation temperature seemed to be around 59^{o}F for both fast germination and high germination rate (Figure 2), which is common during the spring season in central Oregon. Given the prolonged cold period from winter to spring in central Oregon, the germination of ergot bodies would be expected to be longer than 20 days.

The results from this study were obtained from one time study from new ergot bodies collected from infected bluegrasses at COARC. Replication of this experiment with ergot bodies collected from other sources will be very helpful for a better understanding of the biology of this pathogen, and for prediction of the time when ascospores are produced by the sclerotia in the soil.

***Release* of Ascospores and Weather Conditions in Bluegrass Fields**

Soil temperature and air temperature followed a similar pattern, and increased from April 27 to June 30 (Figure 3). The daily variation of soil temperature was generally narrower than the air temperature, ranged from about 40 to 55^{o}F in the end of April and from about 55 to 65^{o}F late June, comparing with air temperature from 30 to 60^{o}F and 40 to 80^{o}F for the two periods, respectively. According to the results obtained on germination of ergot bodies of the pathogen, the soil temperature observed in this bluegrass field was in the lower end of optimal temperature range for ergot germination before June 4. Then after, the soil temperature warmed up into mid and upper range of the optimal temperature range. The air temperature in mid and late June, although varied widely, is generally optimal for infection of the pathogen (Montes-Garcia et al., 2009). The 2011 spring was wet and cold, and rainfalls were recorded regularly during the period from Aril 27 to June 20 (Figure 3). As a result, the flowering of bluegrass in both fields was delayed to around June 13 to June 20, with the grower's field flowering slightly earlier. Release of ascospores was observed in both fields (Figure 4). The beginning of ascospore release was slightly earlier in the grower's field on the Agency Plain than in the field at COARC, but in both fields, ascospores overlapped with the flowering period. Consequently, ergot was observed in both fields (data not shown). The average incidence of infected heads was 4.2%, with average 1.36 ergot bodies on each infected head in the grower's field where Tilt was applied for ergot (data not shown). In the field at COARC where no chemical was applied for ergot (data not shown), the incidence of infected heads (35.1%) and average number of ergot bodies (2.80) on each infected head were much higher.

Levels of Ergot in Plots With and Without Debris Burning

The incidence of infected heads was generally high in all the plots this year (Figure 5). The statistical analysis suggested that the difference between varieties were significant (data not shown) with incidence significantly higher in Volt than that in any other cultivar (Figure 5). The difference between A00-891 and two other varieties, though large, was not statistically significant. Because ergot bodies were distributed into the plots with Volt, it is unclear whether the difference was solely due to higher susceptibility of the cultivar or the higher pathogen inoculum in those plots. The difference between debris burning and no debris burning treatments was insignificant too (Figure 5, result of statistical analysis was not shown).

The Epidemiological Roles of Conidia (honeydew) in the Spread of the Disease in Kentucky Bluegrass

No ergot bodies were observed until July 10, almost 4 weeks after initial inoculation and subsequently, the final levels of ergot were generally low in these plots (Table 1). Although there were more infected heads (seeds) in plots inoculated, the effects of inoculation, rows, and their interaction on the number of infected heads were statistically insignificant (data not shown). Ergot bodies were observed both in un-inoculated rows of inoculated plots and in un-inoculated plots. Since they were not observed early when the ascospores were widely available, the infections were likely to be caused by conidia of the pathogen. We also observed considerable

ergot bodies on ornamental grass plants at least 500 ft away from other grass fields at COARC. These suggested that the conidia (or less likely ascospores) of this pathogen can be transported over a considerable distance. In order to develop an advisory system to guide fungicide application in the future, it is very important to further study what is the main inoculum being transported, conidia or ascospores, and by what means they are transported over distance.

Acknowledgements

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References

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Table 1. Levels of ergot in plots with and without inoculation.

	Treatment	Center row	Other rows
Infected heads			
	No inoculation	0.00	0.13
	Center row inoculated	2.25	0.94
Ergot bodies			
	No inoculation	0.00	0.31
	Center row inoculated	3.50	3.50

Note: The bluegrass plants were transplanted prior to flowering on 5/26. Four replicates were included for each of the two treatments with and without inoculation of the center row in the plots consisting of five 5 ft rows. The center row was inoculated on 6/13 and 6/21 by spraying a suspension of conidia (10^5 conidia/ml) produced on acid PDA in the laboratory and boards were used to prevent drift to other rows during the inoculation.

Figure 1. A photo of the experimental setup for germination of ergot bodies. Ergot bodies were placed on a polyurethane disc in a foam cup with 4 small holes on the side wall to drain excessive water.

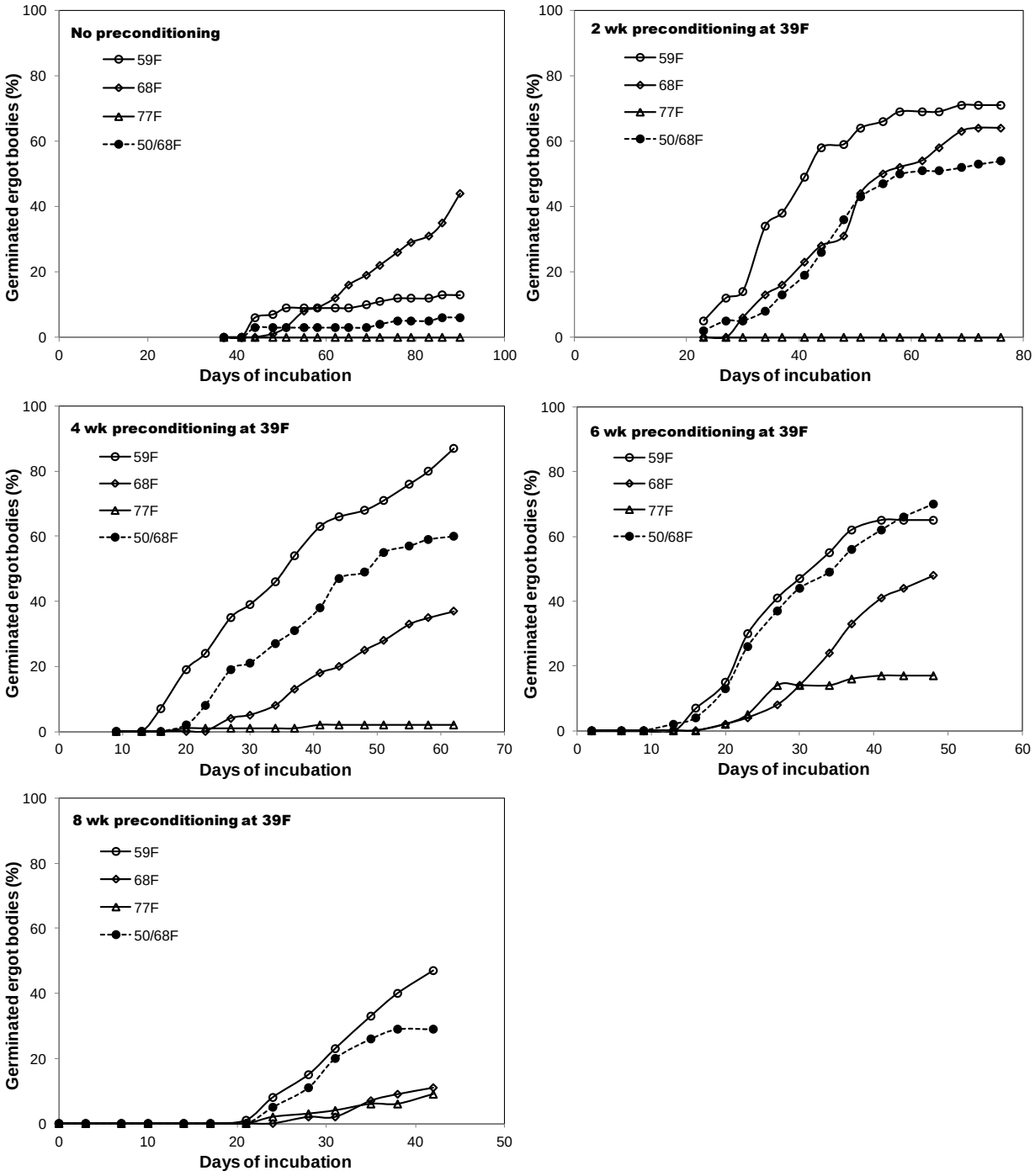

Figure 2. Germination of ergot bodies in laboratory. The sclerotia have been surface sterilized with 2% bleach, rinsed three times with sterilized water and then incubated at 59, 68, 77, and 50/68^{o}F after a preconditioning treatment at 39.2^{o}F for 0, 2, 4, 6 and 8 weeks.

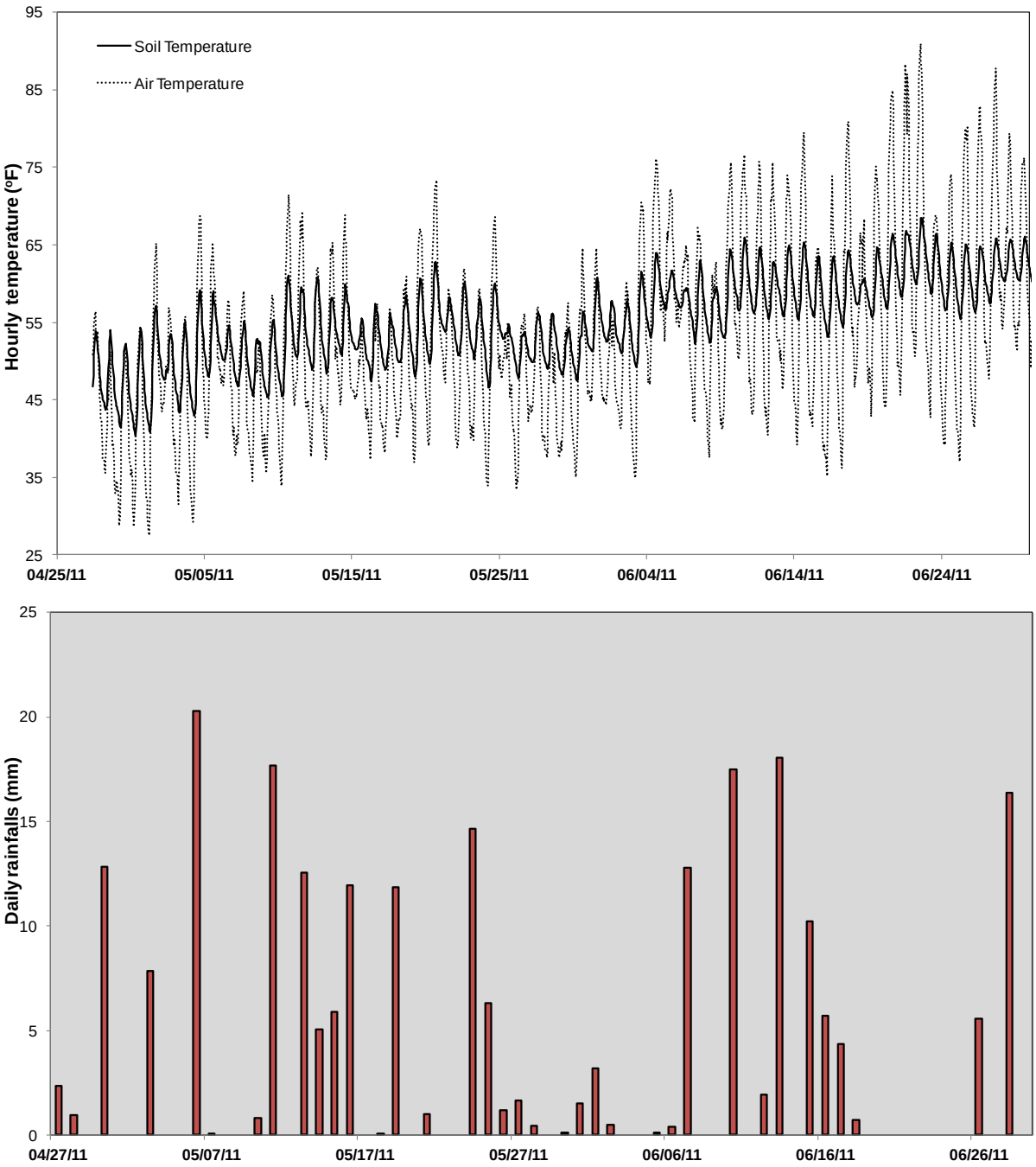

Figure 3. Hourly average soil temperature at 2-inch depth, hourly average air temperature and daily rainfalls (include irrigation) recorded in a Kentucky bluegrass field in central Oregon in 2011.

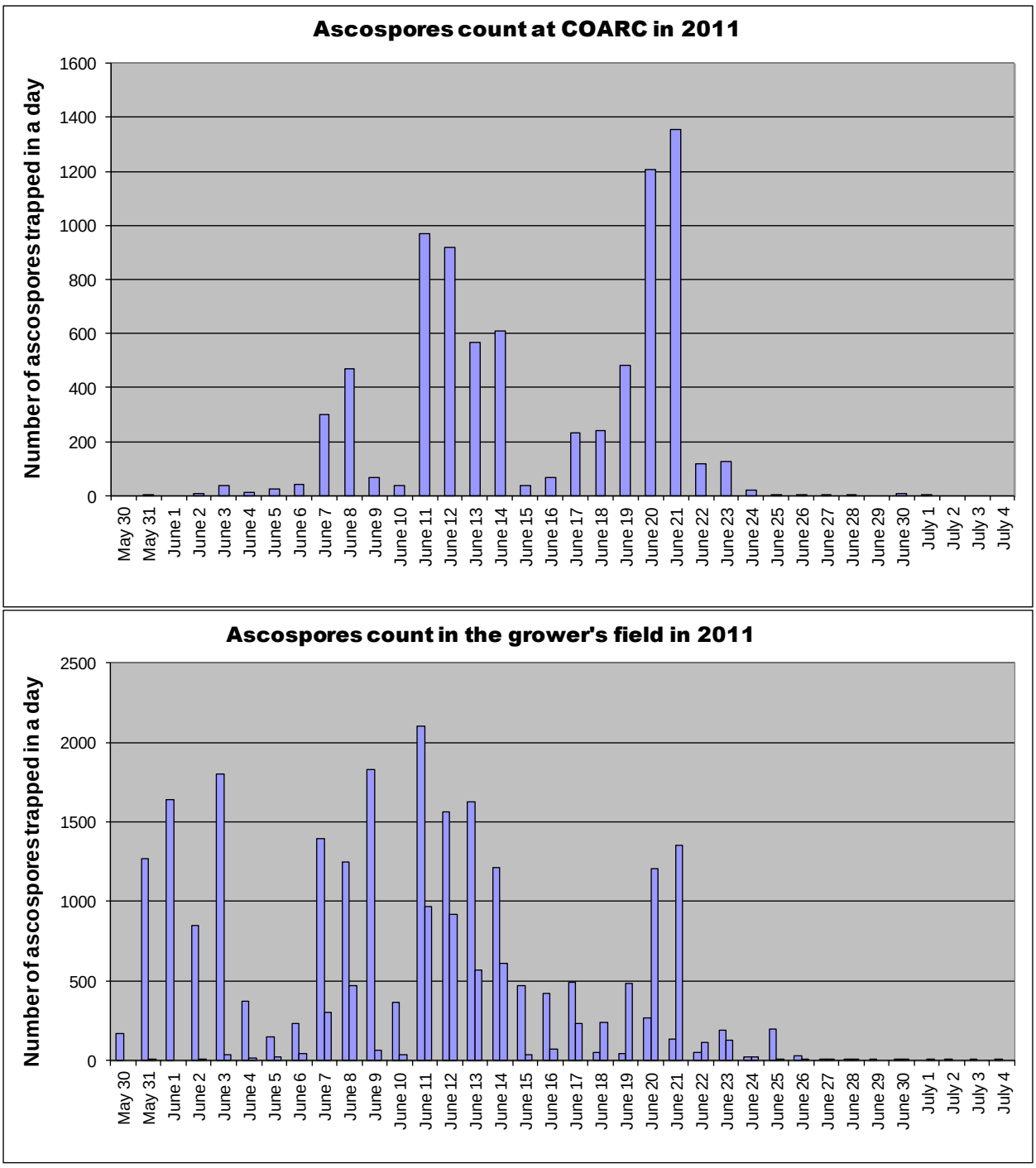

Figure 4. Daily accumulative numbers of ascospores trapped by a 7-day volumetric spore trap in a grower's field on Agency Plain and in a field at Central Oregon Agricultural Research Center, Madras, Oregon. The numbers were adjusted according to the real airflows to reflect the number at an airflow of 10 L/minute.

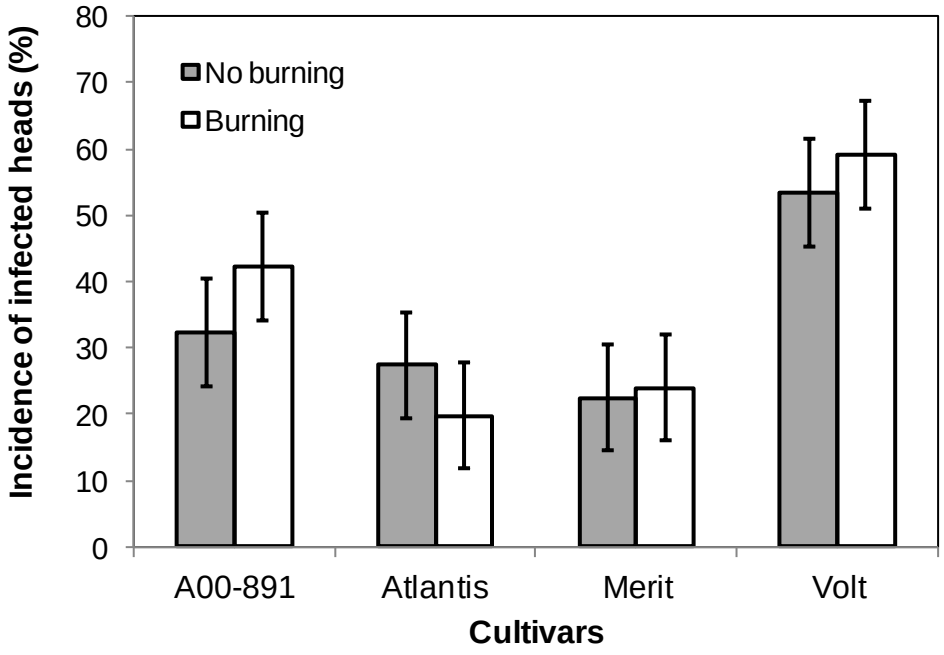

Figure 5. Incidence of infected bluegrass heads (with ergot bodies) in subplots with and without debris burning treatments within the main plots with different cultivars. Two hundred heads were evaluated in each of 4 replicate subplots.