DIVERSITY AND ERGOT INCIDENCE AMONG INSECT POPULATIONS IN KENTUCKY BLUEGRASS AND PERENNIAL RYEGRASS SEED FIELDS IN THE COLUMBIA BASIN

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Introduction

Ergot, caused by the fungus *Claviceps purpurea*, is an important floral disease of perennial ryegrass (PRG, *Lolium perenne*) and Kentucky bluegrass (KBG, *Poa pratensis*) seed crops in the Pacific Northwest (Alderman, 1991). The fungus colonizes unfertilized ovaries, resulting in production of elongated black sclerotia that replace the seed. During infection, asexual spores (conidia) mix with plant sap and exude from florets in what is referred to as the "honeydew" stage of infection.

Ergot honeydew can serve as a food resource for insects such as flies, beetles, wasps, and piercing and sucking insects. When these insects land or feed on the flowers, they may facilitate the insect-mediated secondary dispersal of *C. purpurea*. Based on microscopic examinations, Butler et al. (2001) reported that 75% of flies and 100% of moths collected from KBG fields in central Oregon carried ergot conidia. However, there is limited information on the association of ergot disease and insect abundance in KBG and PRG fields in northeastern Oregon. Furthermore, ergot conidia can be morphologically similar to other fungal spores. Therefore, molecular techniques could aid in identifying and distinguishing *C. purpurea* conidia from other fungal spores in or on insects.

The need to understand the role of insects in ergot dispersal was prompted by growers' concerns and inadequate information about insect vectors. A previous study by Rondon indicated that the majority of insect species in PRG and KBG fields were beneficial ground beetles, rove beetles, and other predatory insects, based on data collected using pitfall traps, sweep nets, and sod samples (Rondon, 2009). The information obtained from the 2008 study was used by Rondon's lab to refine monitoring, trapping, and identification methods in 2009. This effort provided a foundation for understanding the insect population structure in grass seed crops and is the basis for our investigation in 2014 to obtain a better understanding of the potential role that insects may play in ergot dispersal.

The objectives of our study were to: (1) estimate insect abundance in PRG and KBG research trials planted at the Hermiston Agricultural Research and Extension Center (HAREC) in 2009 and in both commercial fields and research trials in 2014; (2) determine whether ergot conidia are associated with insects and whether a correlation exists between insect abundance and ergot incidence; and (3) develop a high-fidelity polymerase chain reaction (HF-PCR) protocol to confirm the presence of *C. purpurea* spores on insects.

Materials and Methods

Survey of insect abundance—2009

Arthropod diversity was monitored in one KBG trial (cv. 'PST 102-68') and one PRG trial (cv. 'Metropolitan') located at HAREC during April and June 2009. Each field was 0.15 acre in size and split into four equal sections to represent replicated plots. Samples from 6 pitfall traps, 10 sweeps with a standard 15-inch sweep net, and 6 sod samples (1 foot in diameter by 4 inches deep) were taken weekly in each section of each field. Insects were collected, sorted, and counted from all samples. Species identifications were made by using identification keys and by comparing sampled insects with voucher specimens held at the Irrigated Agricultural Entomology Program Laboratory at HAREC and at the Oregon State Arthropod Collection at Oregon State University in Corvallis, OR.

Survey of insect abundance and ergot incidence—2014

Insect abundance was monitored from May to June 2014 in four commercial KBG fields (cvs. 'Midnight', 'Arrowhead', 'Brooklawn', and 'Bonaire') and three commercial PRG fields (cvs. 'Pavilion', 'Presidio', and 'TopHat2') located in the Columbia Basin of Washington and Oregon, respectively, and in experimental field plots (0.1 acre) situated in a PRG variety trial located at HAREC. Each commercial field was approximately 125 acres and divided into four equal quadrants representing four replicates. Sampling was conducted weekly in each quadrant using universal black light traps, delta traps, yellow sticky cards, and sweep nets. Ergot incidence in the commercial fields was calculated at crop harvest, based on the number of infected seed heads containing sclerotia out of 100 seed heads collected from each quadrant of field sampled. Correlations between ergot incidence and insect abundance were calculated.

Detection of fungal spores

Insects collected during 2014 were sorted, counted, and stored at −20ºC until preliminary microscopic examination for the presence of ergot spores. Subsamples of 30 insect specimens from each field were used in microscopic studies. Insects were dissected, and fluid from the insect digestive tract was examined under a compound microscope for the presence of ergot conidia. Since this technique is time consuming and could lead to inaccurate identification of fungal spores, a HF-PCR protocol was developed to detect the presence of ergot spores. Whole insect samples were subjected to genomic DNA extraction using a modified CTAB method as described by Zhang et al. (1998). Genomic DNA was used to confirm the presence of ergot spores in insect extracts using the HF-PCR protocol. Ergot specific forward (5'-GCTCTAGACTGCTTTCTGGCAGACC-3') and reverse (5'-CGTCTAGAGGTACCCATACCGGCA-3') β-tubulin primers (Tooley et al., 2001) were used to amplify the target gene. PCR products were visualized on a 1% agarose gel. The subset of positive PCR products were cloned, sequenced, and compared with sequences in GenBank to confirm the identification of fungal isolates belonging to *C. purpurea*.

Table 1. Species composition and average number of insects collected in perennial ryegrass (PRG) and Kentucky bluegrass (KBG) fields between April and June 2009.

Order Common name Genus/species	PRG	KBG
	Average number	
Coleoptera		
Ground beetles		
Bembidion spp.	2.3	29
Amara quenseli	10	2.5
Amara conflata	0.7	1
Rove beetles		
Philonthus fuscipennis	179	0
Hymenoptera		
Parasitoid wasps		
Ichneumonid spp.	1	0
Bracon spp.	22	0
Diptera		
Lesser house fly		
Fannia canicularis	71	0

Results and Discussion

Survey of insect abundance—2009

The 2009 study provided a better understanding of the biodiversity of insects in grass seed crops. Ground beetles (*Bembidion* spp. and *Amara* spp.) were found in both KBG and PRG (Table 1), while rove beetles (*Philonthus fuscipennis*) and parasitoid wasps (ichneoumonids and braconids) occurred only in the PRG field. Similarly, the lesser house fly (*Fannia canicularis*) occurred only in the PRG field. The difference in insect populations between these two crops could be due to differences in crop management practices, habitat suitability, and/or food availability.

Survey of insect abundance and ergot incidence—2014

Dipteran insects were the largest proportion of the insect community in both KBG and PRG seed fields in 2014, comprising 60% of the insects collected (Figure 1). An abundance of dipteran insects was also observed in PRG fields during the 2009 insect survey (Table 1). A significant and positive correlation existed between insect abundance and ergot incidence in PRG fields surveyed in 2014 (Figure 2). However, correlations could not be established in KBG fields because ergot incidence was negligible (data not shown). The reason for the low occurrence of ergot in KBG fields is not known at this time, but spore trap data indicate that very few ascospores were present in these fields (unpublished data).

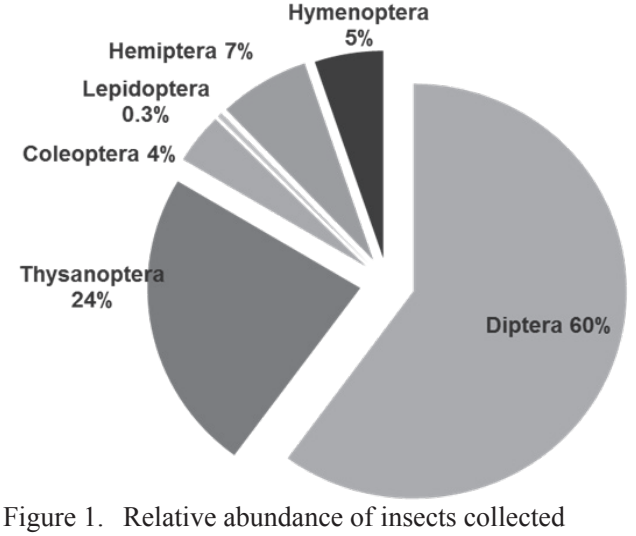

Figure 1. Relative abundance of insects collected from commercial Kentucky bluegrass and perennial ryegrass seed fields during May and June 2014. (No differences in the population structure between the two crops were found; therefore, data were pooled.)

Detection of fungal spores

Initial microscopic examination revealed the presence of ergot conidia in at least 50% of the insect gut subsamples; however, taking into account the similarity between ergot conidia morphology and that of other fungi, we relied on molecular detection for further confirmation. The HF-PCR protocol amplified a 527 base pair product from *C. purpurea*. Using HF-PCR, up to 35% of dipterans (muscid flies) and 27% of moths (noctuid moths) tested positive for ergot, indicating accuracy and sensitivity of the method used. These results were consistent with the findings of Butler et al. (2001), in which moths and flies were most commonly contaminated with ergot spores. Understanding the importance and the mechanism of insect-mediated ergot dispersal may aid in developing new strategies to manage this disease.

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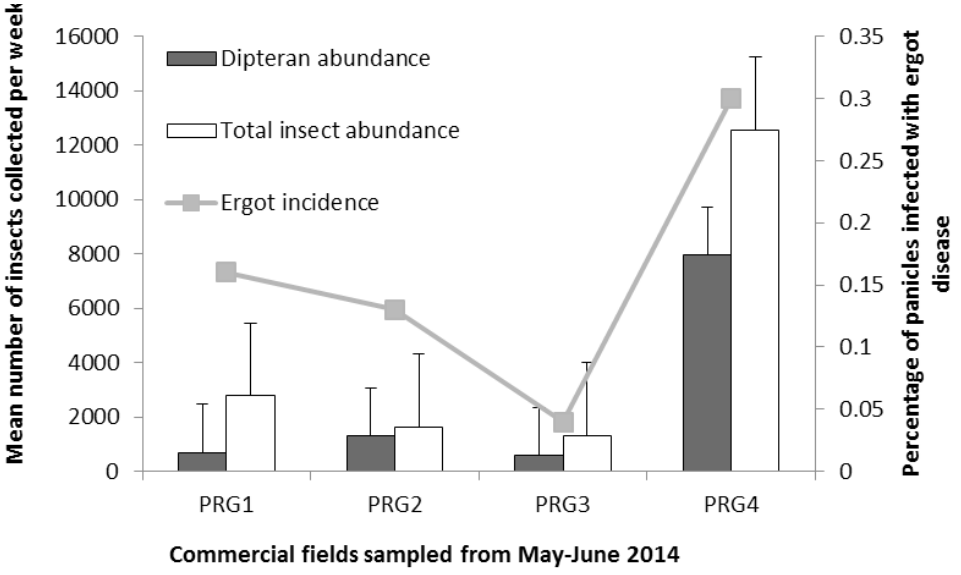

Figure 2. A significant positive correlation ($r = 0.9$, $P < 0.05$) existed between total insect abundance and ergot incidence, as well as between dipteran abundance and ergot incidence ($r = 0.9$, $P < 0.05$) in four commercial perennial ryegrass (PRG) fields during May and June 2014.