UNDERSTANDING BILLBUG SPECIES COMPLEX IN GRASS SEED PRODUCTION SYSTEMS IN WESTERN OREGON

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Introduction

Billbugs (*Sphenophorus* spp.) are complex weevil pests affecting cool-season grasses across the United States (Dupuy and Ramirez, 2016). In the Pacific Northwest (PNW), at least four species of this genus are known to cause significant damage to grass seed crops. The billbug species complex that is commonly found in Oregon grass seed production systems is comprised of the bluegrass billbug (*Sphenophorus parvulus*), Denver billbug (*Sphenophorus cicatristriatus),* orchardgrass billbug (*Sphenophorus venatus confluens*), and *Sphenophorus* sayi (no common name) (Walenta et al., 2004). There are four life stages: egg, larva, pupa, and adult. Adults feed on spring regrowth, chewing through young, folded leaves near the plant crown. As leaves extend, the characteristic damage of paired holes through leaf interiors is observed (Salisbury and Anderson, 2020).

Adult damage is not of major concern, as it has little effect on yield. Billbugs do most of their damage while in the larval stage and can cause significant damage by feeding on stems, roots, and crowns, causing severe discoloration and eventual plant death. Heavy larval feeding compromises the root system, and stems of severely damaged turf break and pull away easily from the soil. Often, a sawdustlike frass is present in hollowed-out stems (Salisbury and Anderson, 2020). Due to the cryptic nature of larvae and similarity of damage symptoms, i.e., weak crowns or presence of sawdustlike frass material, billbug damage in western Oregon grass fields is sometimes misdiagnosed as sod webworm damage.

Bluegrass billbug (*S. parvulus*), the main pest of Kentucky bluegrass grown for seed, was initially thought to be limited to eastern Oregon production systems until the recent report of this species occurring in commercial tall fescue fields in western Oregon at levels causing visible crop damage (Anderson, personal communication). Therefore, monitoring efforts to better understand the billbug species complex occurring in western Oregon are warranted.

The key morphological characteristics that can help distinguish between adults of at least three important billbug species of concern to grass seed crops are presented in Figure 1. The Denver billbug is about 10–12 mm in length and has small, even dimples on the thorax. The orchardgrass billbug is about 7–8 mm long, the thorax has many tiny indentations that look like pinholes, and the wing covers are coarsely grooved. The bluegrass billbug has even dimples covering the thorax and is 5–7 mm in length. No such characteristics exist for larval identification of these three species, which further complicates the understanding of their biology and seasonal phenology, as regional billbug pest complexes can co-occur in the same agroecosystems and are difficult to find during sampling.

Figure 1. Adults (left to right): Denver billbug (*S. cicatristriatus*), orchardgrass billbug (*S. venatus confluens*), and bluegrass billbug (*S. parvulus*). Photo: Babu Panthi, OSU Field Crop Entomology Program.

Materials and Methods

Sampling

Monitoring efforts were conducted in five commercial tall fescue fields, including three in Yamhill County and two in Washington County, OR. Weekly sampling for billbugs was initiated in September 2019 and continued through mid-November. Sampling resumed in January 2020 and continued until seed harvest. Monitoring efforts included nondestructive linear pitfall sampling of ground-active billbug adults and destructive sampling

using soil cores for larval stages in the soil. Two linear pitfall traps (a slit PVC pipe allowing linear movement of insects into a collection cup containing killing agent) were installed at each commercial field site. Specimens collected from pitfall traps were stored in plastic containers, brought to the laboratory, counted, and forwarded to the OSU Field Crops Entomology Lab for confirmation of species identification using molecular techniques.

Sod-soil samples were also collected from each site on a biweekly basis to target the presence of immature stages. Samples were taken with a shovel, collecting at least a 4-inch (total) sample according to sampling techniques modified from Walenta et al., 2004. Sod samples were subjected to either Berlese funnels or manual dissections in the laboratory.

Molecular studies

DNA-based identification methods were employed, adapted from Duffy et al. (2018), to confirm the species identification of billbugs collected in western Oregon. PI Walenta collected Denver billbug samples from a commercial Kentucky bluegrass field in eastern Oregon to be included in this study for a comparative analysis.

Following manufacturer protocols, genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Valencia, CA) from whole-body homogenizations of the thorax and abdomen for three species: *S. venatus*, *S. parvulus*, and *S. cicatristriatus*. DNA was quantified using a ThermoScientific NanoDrop 2000 spectrophotometer. Three loci—COI, 18S, and ITS2—covering mitochondrial (mtDNA), ribosomal (rDNA), and nuclear ribosomal (nrDNA) DNA, respectively, were amplified using polymerase chain reaction (PCR). The PCR products upon cleanup using Qiagen kits were submitted to the Center for Genome Research and Biocomputing (CGRB) at OSU for Sanger sequencing. The NCBI website's BLAST search function was used to identify insect DNA sequences based on their similarity to archived sequences.

Results and Discussion

This study revealed that the bluegrass billbug and orchardgrass billbug were found to co-occur in western Oregon tall fescue fields, as indicated by the number of adult captures in the pitfall traps at all five field sites in 2019–2020 (Figure 2). The first adult beetle captures corresponded to *S. parvulus* in the pitfall traps and occurred in late February 2020 at two field sites (Figure 2), indicating resumption of bluegrass billbug adult foraging activity after the overwintering period.

The first and only larva capture occurred in late May at only one sampling site, corresponding to the bluegrass billbug, based on its 100% similarity to the sequence reads of *S. parvulus* isolate AD01 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence corresponding to GenBank Accession (MG385047). Weekly sampling efforts targeted for larval and pupal stages were not able to detect these life stages throughout the crop harvest period.

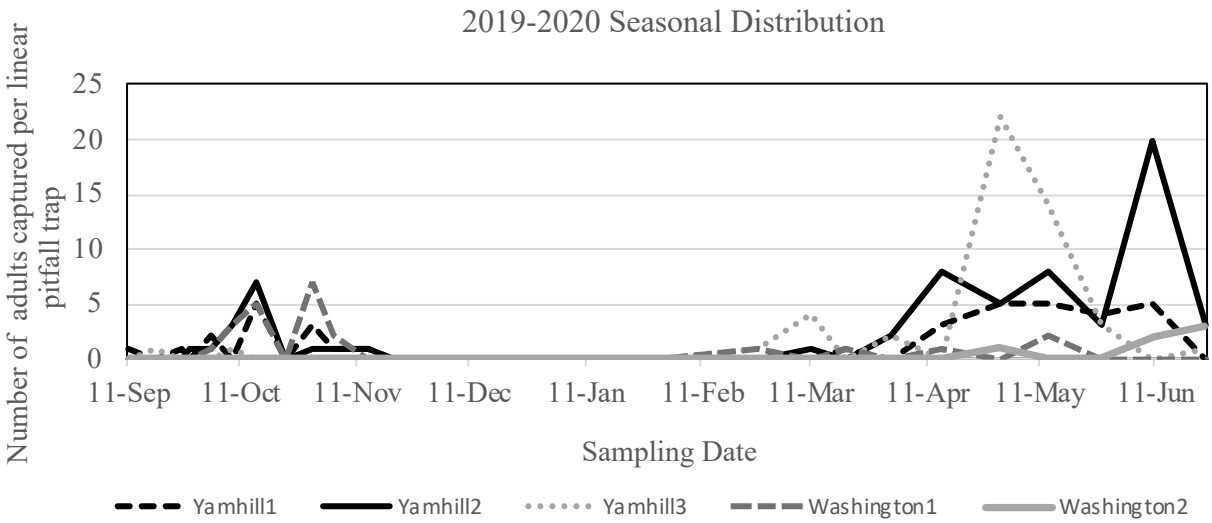

Figure 2. Cumulative adult capture of the bluegrass billbug (*S. parvulus)* and orchardgrass billbug *(S. venatus*) in linear pitfall traps each week at five commercial tall fescue seed fields in western Oregon, 2019–2020.

Additional scouting efforts in early August through September 2020 in commercial fine fescue seed fields (not included in this study) revealed the presence of larval and pupal stages corresponding with 100% identity to the orchardgrass billbug isolate AD06 5.8S ribosomal RNA gene and internal transcribed spacer 2, partial sequence GenBank Accession (MG385050).

The sequence read based on CO1 primers obtained from Denver billbug corresponded to the CO1 region of *Sphenophorus* spp. BYU-CO246 mitochondrion, complete genome with 100% identity to GenBank Accession (GU176342.1).

The sequence data for all loci—COI, 18S, and ITS2—are being processed for submission to the GenBank for the billbug species complex found in Oregon surveys for future referencing. The data collected during 2019–2020 on insect phenology of the bluegrass billbug is indicative of only one generation per year in western Oregon. This closely aligns with the earlier phenology model proposed for the bluegrass billbug in eastern Oregon by Rondon and Walenta (2011).

References

Duffy, A.G., G.S. Powel, J.M. Zaspel, and D.S. Richmond. 2018. Billbug (Coleoptera: Dryopthoridae: *Sphenophorous* spp.): Seasonal biology and DNA-based life stage association in Indiana turfgrass. J. Econ. Entom. 111:304–313.

Dupuy, M.M. and R.A. Ramirez. 2016. Biology and management of billbugs (Coleoptera: Curculionidae) in turfgrass. J. Integr. Pest Manage. 7:1–10.

Rondon, S.I. and D.L. Walenta. 2011. Elucidating the biology of the bluegrass and Denver billbugs in NE Oregon. In W.C. Young III (ed.). *2010 Seed Production Research Report.* Oregon State University, Ext/CrS 130.

Salisbury, S.E. and N.P. Anderson. 2020. Grass seed pests. In C.S. Hollingsworth (ed.). *Pacific Northwest Insect Management Handbook*. Oregon State University. https://pnwhandbooks.org/insect

Walenta, D.L., S. Rao, C.R. McNeal, B.M. Quebbeman, G.C. Fisher. 2004. *Sphenophorus* spp., a complex billbug community infesting Kentucky bluegrass seed fields in the Grande Ronde Valley of northeastern Oregon. In W.C. Young III (ed.). *2003 Seed Production Research Report*, Ext/CrS 124.

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