Unraveling Billbug Seasonal Ecology to Improve Management: Developing a DNA-Based Larval Identification Tool

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Objectives:

1. Identify key regions in billbug rDNA sequences that can be used to identify and differentiate major billbug pest species.
2. Create species-specific DNA primers for these same rDNA regions that will allow for rapid and dependable identification of field-collected billbug larvae

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Billbugs are increasingly being recognized as a serious threat to golf course turf across the United States. The larvae of this diverse group of insect species (a species complex) damage both cool- and warm-season turfgrasses by feeding on or inside the stems, crowns, roots, stolons, and rhizomes. Recent expansions in the range of several billbug species, possibly driven by suburban development and increasing interstate movement of turfgrass sod, have resulted in a national collage of billbug species assemblages.

The resulting variation in seasonal life histories, behavior, and ecology that often accompany such novel species interactions have challenged management schemes in many regions. Although adult monitoring can be used to estimate billbug species composition and track adult activity, it is the larvae that are primarily responsible for damage. Unfortunately, the larval stages of these insects cannot presently be identified to species.

The long-term goal of this project is to clarify the seasonal ecology of the billbug species complex and elucidate new management opportunities. The first phase of this research is focused on developing a DNA-based billbug larval identification tool that will facilitate the basic, regional studies of billbug seasonal ecology that are needed to improve management.

The tandemly arrayed ribosomal RNA multigene family is a common target of efforts to differentiate closely related species because of useful features of its sequence organization and evolution. Portions of the rDNA coding sequences are highly conserved even between distantly related species, allowing the application of "universal" primers for amplification from any species. The non-coding rDNA spacer sequences, however, can be highly variable in length and sequence between closely related species making them particularly useful for species-diagnostic assays.

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Several hundred billbug adults and larvae were collected from various locations across the U.S. Specimens include all known billbug pest species: bluegrass billbug (*Sphenophorus parvulus*), lesser billbug (*S. minimus*), unequal billbug (*S. ineaqualis*), Denver or rocky mountain billbug (*S. cicatristriatus*), hunting billbug (*S. venatus*), and Phoenix billbug (*S. phoeniciensis*). Adult specimens were identified to species using classic morphological characteristics. After the identity of adult specimens was confirmed, several regions (ITS1, ITS2 and CO1) of ribosomal DNA were extracted, amplified, and sequenced. Sequences were compared to determine which ones were most useful for differentiating billbug species.

The ITS2 region (internal transcribed spacer region 2) between the 5.8s and 18s rDNA sequences was the first target of our investigation. Based on the size and sequence of ITS2, *S. parvulus* and *S. minimus* may be differentiated from each other based on the size (number of base pairs) of the region alone. Furthermore, ITS2 allowed differentiation of these two species from all other species examined. However, ITS2 did not provide the differences in size or sequence necessary to dependably differentiate all of the billbug species examined.

Likewise, CO1, the second rDNA region examined, did not prove at all useful in differentiating billbug species as the size and sequence of this region was identical for all species. ITS1, which is currently being examined, will likely provide further resolution between species as the ITS1 sequence for all species examined to date varies significantly in size.

Summary Points

- A DNA-based billbug larval identification tool could provide researchers with the means to gain a more complete understanding of the seasonal biology of the billbug pest complex and provide a science-based foundation for improving management programs.
- The ITS2 region of the rDNA will allow differentiation between *S. parvulus* and *S. minimus* as well as differentiation of these two species from all other species examined.
- Although CO1 was not useful for differentiating any of the species examined, ITS1 will likely provide further resolution between species as the ITS1 sequence for three of the five species examined to date varies significantly in size.
- When complete, DNA primers can be developed for use in a multiplex PCR system that will allow accurate identification of field collected billbug larvae, facilitate studies of billbug seasonal ecology, and improve billbug management.