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 (the billbug species complex) damage both warm- and cool-season turfgrass by feeding on or inside the stems, crowns, roots, stolons, and rhizomes. Recent expansions in the range of several billbug species, possibly driven by increasing interstate movement of turfgrass sod, have resulted in a nationwide collage of billbug species assemblages. The resulting variation in seasonal life histories, behavior, and ecology that often accompany such novel species interactions have made satisfactory management difficult to achieve in many regions.

Although adult monitoring can be used to estimate billbug species composition, insight into billbug larval population dynamics is needed to improve management strategies and identify which species are actually responsible for damage to turfgrass. Because the damaging larval stages of these insects cannot presently be identified to species, the seasonal population dynamics of many common billbug pests remains unresolved, leaving researchers and superintendents with little scientific foundation on which to base and refine management programs.

The long-term goal of this project is to clarify billbug seasonal ecology and elucidate new management opportunities. In working toward this goal, 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.

Several hundred billbug adult and larval specimens were collected from various locations across the U.S. Specimens include all known billbug pest species; the bluegrass billbug (*Sphenophorus parvulus*), the lesser billbug (*S. minimus*), the unequal billbug (*S. ineaqualis*), the Denver or Rocky Mountain billbug (*S. cicatristriatus*), the hunting billbug (*S. venatus*), and the Phoenix billbug (*S. phoeniciensis*). Adult specimens are identified to species using classic morphological characters. After the identity of adult specimens is confirmed, several regions of the rDNA are extracted, amplified, and sequenced to determine which of these regions are most useful for differentiating billbug species.

Billbug adult (left) and larva (right). Although adults can be identified relatively easily based on morphological characters, larvae are much more difficult to accurately identify.

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. Concerted evolution acting on rDNA arrays maintains sequence homogeneity within species as it drives differentiation between species, a pattern that explains the utility of rDNA for species-diagnostic assays.

The ITS2 region (internal transcribed spacer region 2) between the 5.8s and 18s ribosomal DNA sequences has been the first target of our investigation. Based on the size and sequence of ITS2, results to date indicate that this region will allow differentiation between *S. parvulus* (338 base pairs) and *S. minimus* (463 base pairs) based on the size (number of base pairs) of the region alone. Furthermore, ITS2 will allow us to dependably differentiate these two species from *S. cicatristriatus*, *S. venatus*, and *S. inaequalis*, all of which share an ITS2 region of identical size (348 base pairs) and sequence.

However, because ITS2 does not provide the differences in size or sequence necessary to dependably differentiate all of the billbug species of concern, efforts are being re-directed to examine several other promising regions within the ribosomal RNA multigene family (ITS1, CO1, and IGS).

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 this emerging pest complex on a region-by-region basis, leading to improved management programs.
- The ITS2 region of the rDNA multigene family will allow differentiation between *S. parvulus* (338 base pairs) and *S. minimus* (463 base pairs) based on the size (number of base pairs) of the region alone.
- ITS2 will also allow us to dependably differentiate these two species from *S. cicatristriatus*, *S. venatus*, and *S. inaequalis*, all of which share an ITS2 region of identical size (348 base pairs) and sequence.
- Because ITS2 does not provide the differences in size or sequence necessary to dependably differentiate all billbug species of concern, future efforts will focus on other regions within the ribosomal RNA multigene family (ITS1, CO1, and IGS).