DEVELOPMENT OF EFFECTIVE MONITORING TECHNIQUES FOR SHARPSHOOTERS AND THEIR PARASITOIDS.

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Reporting Period: The results reported here are from work conducted October 9, 2010 to October 9, 2011.

ABSTRACT

Gonatocerus morgani, *Gonatocerus morrilli*, and *Gonatocerus t*riguttatus have been reared and released by the Pierce's Disease program at sites throughout Southern California and the southern Central Valley since 2000. However, data concerning the extent of released species populations, the effects of parasitism by native competitors, and the host preferences of the parasitoids involved is still needed, even though data presented in the most recent CDFA report (2010) demonstrates the effectiveness of the release program. In conjunction with his research in comparative and functional genomics of *Xylella fastidiosa (Xf)*, D. Cooksey has developed a multiplex PCR system for the simultaneous identification of *Xf* strains (Hernandez-Martinez *et al.*, 2006). D. Morgan, an expert in the biology, ecology, systematics, and identification of the host (Son *et al.*, 2009) as well as the parasitoid species targeted in this study, is the supervisor of the release program. C. LeVesque directs a high throughput testing program for citrus Huanglongbing disease that employs high resolution melting curve analysis as developed by Lin *et al.*, 2011. The development of the proposed high resolution melting (HRM) real-time PCR system will greatly enhance the data acquisition of the CDFA parasitoid release biocontrol program which will assist in assessing the efficacy of the ongoing sharpshooter egg parasitoid strategy.

LAYPERSON SUMMARY

In order to efficiently use biological control agents it is essential to have the capacity to identify the parasitoid species, host species and the extent of parasitism. These parameters must be known in order to evaluate the effectiveness of the control strategy. The current method used in the glassy-winged sharpshooter (GWSS) biological control program relies on identification of eclosed parasitoids after long incubations under artificial conditions. Often the parasitoids do not survive. It would greatly facilitate the development of the release program if an accurate and rapid method for identification of the eggs of sharpshooter species, determining whether eggs are parasitized, and by which parasitoid species, were available. The proposed single-step real-time HRM PCR assay for sharpshooters and their parasitoids will provide such a tool and will significantly enhance the reporting of GWSS parasitism.

INTRODUCTION

The post-release collection and incubation of field-collected glassy-winged sharpshooter (GWSS) eggs is currently the only methodology available for monitoring the GWSS biocontrol program. Since the eggs are removed from the field before development has been completed, the possibility of further parasitism is eliminated and, therefore, parasitism rates are underestimated. In addition, because optimal incubation conditions vary for each parasitoid species, significant developmental mortality can occur during the two-week or longer incubation period needed for wasps and GWSS to eclose. This results in some species being significantly underreported. If there is no sharpshooter emergence, there currently is no economical method for identifying whether eggs are from GWSS or the native smoke tree sharpshooter (STSS). Therefore, it is essential that a more efficient method for monitoring biological control activity be developed if we are to have more accurate, timely, and economic reporting of GWSS parasitism.

Accurate reporting of GWSS parasitism will be accomplished with the development of a single-step HRM real-time PCR assay for sharpshooters and their parasitoids. The identity of the species of host, GWSS or STSS, and its parasitoids can simultaneously be determined with this method within half a day of collection, rather than two weeks. In addition, because the wasp pupal and sharpshooter egg casing can be analyzed, old egg masses should be able to be used after wasp eclosion. The refinement of control strategies by determining the effectiveness of the different parasitoid species in the various environments encompassed in the current range of GWSS will lead to better suppression of GWSS populations.

OBJECTIVES

1. Develop primer pairs that can be used in a multiplex high resolution melting curve analysis real-time PCR system for each species of sharpshooter and parasitoid.
2. Through the use of degenerate primers, clone the target genes from those species of parasitoid for which there is no sequence data available.
3. Determine the limits of detection of each species of sharpshooter and parasitoid. Based on other studies, we are confident we will be able to detect developing parasitoid embryos in sharpshooter eggs. We hope to be able to determine

the both the host and parasitoid species from sharpshooter egg cases from which the parasitoids have eclosed by amplifying the layer of cells which remain in the parasitoid egg (Oda and Akiyama-Oda, 2008).

RESULTS AND DISCUSSION
Degenerate primers were designed to target the COI gene in each of the wasp species, while a separate pair of primers were designed to target the STSS and GWSS COI genes (**Table 1**). These were used to clone ~150 bp fragments from each species to be used as control plasmids for HRM (**Figure 1**).

While HRM primers and reaction conditions are being optimized, GWSS eggs are being collected which have been parasitized by the different wasp species included in the study. These eggs are obtained by setting up fresh egg masses with recently eclosed female wasps. The GWSS eggs are dissected from the leaf tissue at specific intervals after the wasps have been observed ovipositioning.

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FUNDING AGENCIES

Table 1. Primer sequences where R = A,G; K = G,T; and W = A,T.

Primer Name	Sequence
WP-COI-F	5'-CATAGAGGWCCWTCWGTAGATTTATC-3'
WP-COI-R	5'-GATCAACAAAATAARGTWAKTTTTTC-3'
SS-COI-F	5'-GCAACTATAATTATTGCAGTACC-3'
SS-COI-R	5'-GATCATATTATTGAAATTGATATA-3'

Majority   GAACTGGATGAACTGTTTATCCTCCTTTATCAATAAATTTATCTCATAGAGGACCATCXGTAGATTTATCAATTTTTTCT

         330   340   350   360   370   380   390   400

Gonatocerus ashmeadi COI.seq   GAACTGGTTGAACTGTTTATCCTCCTTTATCAATAAATTTATCTCATAGAGGACCTTCAGTAGATTTATCTATTTTTTCA   222
Gonatocerus incomptus COI.seq   GAACTGGATGAACTGTTTATCCTCCTTTATCATCAAATTTATCTCATAGAGGACCTTCTGTAGATTTATCAATTTTTTCT   222
Gonatocerus morgani COI.seq   GAACTGGATGAACTGTTTATCCTCCTTTATCAATAAATTTATCTCATAGAGGACCATCGGTAGATTTATCTATTTTTTCT   222
Gonatocerus morrilli COI.seq   GAACAGGATGAACAGTTTATCCTCCATTATCAATAAATTTATCTCATAGAGGACCATCAGTAGATTTATCAATTTTTTCT   400
Gonatocerus novifasciatus COI.seq   GTACTGGATGGACAGTTTATCCACCTTTATCTTCAAATTTATCCCATAGAGGTCCATCTGTAGATTTATCAATTTTTTCT   222
Gonatocerus triguttatus COI.seq   GTACAGGATGAACAGTTTATCCTCCTTTATCAATAAATTTATCTCATAGAGGTCCATCTGTAGATTTATCAATTTTTTCT   222
Gonatocerus walkerjonesi COI.seq   GAACTGGATGAACTGTTTATCCTCCTTTATCAATAAATTTATCTCATAGAGGACCATCAGTAGATTTATCTATTTTTTCT   222

Majority   TTACATATTGCTGGAGCTTCTTCAATTATAGCTTCAATTAATTTTATTTCTACTATTATTAATATAAGAATATATAAAAT

         410   420   430   440   450   460   470   480

Gonatocerus ashmeadi COI.seq   TTACATATTGCTGGTGCATCATCAATTATAGCTTCAATTAATTTTATTTCTACTACAATTAATATAAAAAATATATAAAAT   302
Gonatocerus incomptus COI.seq   TTACATATTGCTGGAGTTTCTTCAATTATAGCATCAATTAATTTTATTGTATCAATTACTAATATAATTTATTTATAAAAT   302
Gonatocerus morgani COI.seq   TTACATATTGCTGGAGCATCTTCTATTATAGCTTCAATTAATTTTATTTCTACTATTATTAATATAAAAAATATATAGAAT   302
Gonatocerus morrilli COI.seq   TTACATATTGCTGGTGCTTCTTCTATTATAGCTTCAATTAATTTTATTTCTACTACTATTAATATAAGAATATATAGAAT   480
Gonatocerus novifasciatus COI.seq   TTACATATTGCTGGAATTTCTTCAATTATAGCATCAATTAATTTTATTGTATCAATTGTTAATATAATTTATTTATAAAAT   302
Gonatocerus triguttatus COI.seq   TTACATATTGCTGGAGCATCATCAATTATAGCTTCAATTAATTTTATTTCTACTATTATTAATATAAGAATATATAAAAT   302
Gonatocerus walkerjonesi COI.seq   TTACATATTGCTGGAGCTTCATCAATTATAGCTTCAATTAATTTTATTTCTACTATTATTAATATAAGAATATATAAAAT   302

Majority   AGAAAAGTAACATTATTTTGTTGATCTTTAATATTAACAGCAATTTTATTATTATTATCATTACCAGTATTAGCTGGAG

         490   500   510   520   530   540   550   560

Gonatocerus ashmeadi COI.seq   AGAAAAGTTACATTATTTTGTTGATCTTTAATATTAACAGCAATTTTATTATTATTATCATTACCAGTATTAGCTGGAG   382
Gonatocerus incomptus COI.seq   AGAAAAATTAACGTTATTTTGTTGATCATTATTATTAACTTCAATTTTATTATTATTATCTTTACCAGTATTAGCTGGAG   382
Gonatocerus morgani COI.seq   AGAAAAGTTACATTATTTTGTTGATCATTAATATTAACAGCAATTTTATTATTATTATCATTACCTGTATTAGCTGGAG   382
Gonatocerus morrilli COI.seq   AGAAAAGTAACATTATTTTGTTGATCATTAATATTAACAGCAATTTTATTATTATTATCATTACCAGTATTAGCTGGAG   560
Gonatocerus novifasciatus COI.seq   AGAAAAATTAACATTATTTTGTTGATCTTTATTATTAACATCAATTTTATTATTATTATCTTTACCTGTATTAGCAGGTG   382
Gonatocerus triguttatus COI.seq   AGAAAAGTTACATTATTTTGTTGATCTTTAATATTAACAGCAATTTTATTATTATTATCATTACCAGTTTTAGCTGGAG   382
Gonatocerus walkerjonesi COI.seq   AGAAAAGTAACATTATTTTGTTGATCTTTAATATTAACAGCAATTTTATTATTATTATCATTACCAGTITTAGCTGGAG   382

Figure 1. Alignment of *Gonatocerus* COI sequences using ClustalW (Slow/Accurate, IUB) with sequence differences boxed and primers indicated by arrows: