DEVELOPMENT OF EFFECTIVE MONITORING TECHNIQUES FOR SHARPSHOOTERS AND THEIR PARASITOIDS.

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ABSTRACT
Assessing the efficacy of the ongoing sharpshooter egg parasitoid biocontrol program is the focus of this project. Since 2000 *Gonatocerus morgani*, *G. morrilli*, and *G. triguttatus* have been reared and released by the program at sites throughout Southern California and the southern Central Valley. While data presented in the most recent CDFA report (2009) demonstrates the effectiveness of the release program, more data concerning the extent of released species populations, the effects of parasitism by native competitors, and the host preferences of the parasitoids involved is needed. D. Cooksey has developed a multiplex PCR system for the simultaneous identification of *Xylella fastidiosa (Xf)* strains (Hernandez-Martinez *et al.*, 2006), in conjunction with his research in comparative and functional genomics of *Xf*. As Supervisor of the release program, D. Morgan is 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. C. LeVesque has developed a high throughput testing program for citrus Huanglongbing disease that employs high resolution melting curve analysis. The development of the proposed multiplex high resolution melting real-time PCR system will greatly enhance the data acquisition of the CDFA parasitoid release biocontrol program.

LAYPERSON SUMMARY
Glassy-winged sharpshooter (GWSS) populations are partially controlled by biological control agents. It is essential to have the capacity to identify the parasitoid species, host species and the extent of parasitism in order to evaluate the effectiveness of the control strategy. The current methods rely on identification of eclosed parasitoids after long incubations under artificial conditions which many do not survive. An accurate and rapid method for identification of the eggs of sharpshooter species, determining whether eggs are parasitized, and by which parasitoid species, would greatly facilitate the development of the release program. A single-step multiplex real-time PCR assay for sharpshooters and their parasitoids is such a method, and its development will significantly enhance the reporting of GWSS parasitism.

INTRODUCTION
The only methodology currently available for post-release monitoring of glassy-winged sharpshooter (GWSS) parasitoids involves the collection and incubation of field-collected GWSS eggs. Since the eggs are removed from the field before development has been completed, the possibility of further parasitism is eliminated. Therefore, parasitism rates are underestimated. 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, resulting in some species being significantly underreported. No economical method for identifying whether eggs are from GWSS or the native smoke tree sharpshooter (STSS) is currently available. Therefore, a more efficient method for monitoring biological control activity is essential if we are to have more accurate, timely, and economic reporting of GWSS parasitism.

Development of a single-step multiplex high resolution melting (HRM) curve real-time PCR assay for sharpshooters and their parasitoids will allow for accurate reporting of GWSS parasitism. This method can identify the species of host, GWSS or STSS, and its parasitoids simultaneously within half a day of collection, rather than two weeks. In addition, old egg masses should be able to be used after wasp eclosion, as the pupal and sharpshooter egg casing can be analyzed. Determining the effectiveness of the different parasitoid species in the various environments encompassed in the current range of GWSS will facilitate the refinement of control strategies and lead to better suppression of GWSS.

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

Because the available sequences proved unsuitable for the development of satisfactory HRM primers we have used the iCODEHOP program of the University of Washington (Rose *et al.*, 1998), in conjunction with the Lasergene suite of sequence analysis programs, to design degenerate primers targeting a number of genes characterized in various insect species (**Table 1**). Amplification parameters are being developed using the Epicentre Biotechnologies (Madison, WI) FailSafe™ PCR PreMix Selection Kit. PCR products are being cloned using the pGem T-Easy (Promega Corporation, Madison, WI) and the TOPO TA (Invitrogen Corporation, Carlsbad, CA). Cloned PCR products are being sequenced by the Genomics Core sequencing service of the Institute for Integrative Genome Biology on the U.C. Riverside campus.

Table 1. All sequences are 5' to3'. The consensus portions of primers are in uppercase letters while the degenerate portions are in lower case. R = A,G; Y = C,T; M = A,C; K = G,T; S = G,C; W = A,T; H = A,C,T; B = G,T,C; V = G,C,A; D = G,A,T; and N = A,C,G,T.

Gene	Primer	Sequence	Reference
Antennapedia	AP-1CHBF	TGCCATACCCAAGATTCccnccntayga	Hoskins *et al.*, 2007
	AP-1CHCR	CAGGAGGCGTACACAACTGgytgytgytgyt	
	AP-2CHDF	AAGTGCATCAAAACCATCATCAyatggsnatgt	
	AP-2CHER	TTCCAGGGTTTGGTATCTGgtrtangtytg	
Cryptochrome	CC-1CHCF	GGAATAAATAAAATATGTTTCGAACAAgaytgygarcc	Yuan *et al.*, 2007
	CC-1CHDR	TCCACGGTATGCAGGAACatytgrtangt	
KAAT1/CAATCH1	KCAT-1CHAF	GGGCCTGGGCAACGTntggmgnttyc	Castagna *et al.* ,1998
	KCAT-1CHER	CCACGTAGGGGAAGATGgcnarraarta	
	KCAT-2CHEF	GCCTACTTCCTGGCCATCttyccntayrt	
	KCAT-2CHGR	AGAAGTCGAACTTGGCGATGryrtcnggrta	
Maltase	MAL-1CHAF	TGGCAGCACGGAAACTTCtaycarrtnta	Russell *et al.*, 2009
	MAL-1CHDR	CCGGGTTCCGGTAGTTCarrtcngsytg	
V-ATPase c	VATPc-1CHAF	TGTCCGACGACCTGggnaarytnga	Merzendorfer *et al.*, 2000
	VATPc-1CHCR	GTGGTCAGGTATTCGGAGTCCwrdatraartg	
	VATPc-2CHCF	GCTTCCAGTGGGATATGgcnaartaycc	
	VATPc-2CHER	CATCAGCTTGGTCATCTCGttyttnccngc	
Vitellogenin	VIT-1CHDF	CCAAGAACAAAAACAAGGTCTGTTCmgnaaratgga	Meng *et al.*, 2006
	VIT-1CHFR	CTGGTTTATGGGCGGTAccngtccaytc	
	VIT-2CHGF	CTGGTAAACAAGGTCCAATTTACaargcngarac	
	VIT-2CHJR	TCGGATTTTGGCATGTTGttnggrttytg	
	VIT-3CHNF	CGCCCATAACTTCTACCCAACncayatgtayg	
	VIT-3CHQR	GCCACTCTCACTTCGTATGGTtcngcngtrtt	

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