BIOLOGICAL CONTROL OF GLASSY-WINGED SHARPSHOOTER IN CALIFORNIA

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INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae: Cicadellinae), is native to the southeastern states of the USA. As an exotic invader, GWSS has become extremely pestiferous in southern California where it is thought to have established around 1990 (Sorensen and Gill, 1996). GWSS has also successfully invaded Tahiti, [established 1999 (Cheou, 2002)], and Hawaii [established 2004 (Hoover, 2004)]. Within California, GWSS has now become established in the counties of Los Angeles, Orange, Riverside, San Bernardino, San Diego, Ventura, and within portions of Butte, Contra Costa, Fresno, Imperial, Kern, Sacramento, Santa Barbara, Santa Clara, and Tulare counties (CDFA, 2003). Extraordinary population growth has occurred following GWSS's successful establishment in California. This has been facilitated, in part, by a lack of co-evolved natural enemies coupled with irrigation of agricultural and urban areas in desert habitats normally too dry to support GWSS populations (Hoddle, 2004a). GWSS is an economically important pest that vectors *Xylella fastidiosa* (Wells *et al*.). This xylem-dwelling bacteria causes scorch-like disease in susceptible plants which can result in millions of dollars of damage to agriculture production and its related infrastructure. The presence of *Xylella* in Tahiti and Hawaii is currently unknown. It is possible the bacterium has been introduced to these South Pacific Islands by means of the importation of ornamental plants from areas in the Americas where *Xylella* is native. These potential silent *Xylella* reservoirs may harbor bacteria without expressing disease symptoms and, with the arrival of a vector such as GWSS, pathogen transmission to susceptible host plants could conceivably occur.

FOREIGN EXPLORATION EFFORTS FOR GWSS NATURAL ENEMIES

In the southeastern USA and northeastern Mexico, GWSS eggs are parasitized by several species of mymarid and trichogrammatid parasitoids. *Gonatocerus ashmeadi* Girault, *G. triguttatus* Girault, *G. morrilli* Howard, and *G. fasciatus* Girault, all Mymaridae, are the most common natural enemies associated with *H. coagulata* eggs in its home range (Triapitsyn et al., 1998; Triapitsyn and Phillips, 2000). In an effort to use natural enemies to control GWSS populations in southern California, *G. ashmeadi*, *G. morrilli*, *G. triguttatus*, and *G. fasciatus* have been imported from the home range of GWSS, cleared through quarantine, and introduced into urban and agricultural areas. These four parasitoids join resident populations of *G. ashmeadi* and *G. morrilli*. *G. ashmeadi* has been in California since the late 1970s and were probably introduced to southern California on plants bearing parasitized GWSS eggs, and most likely established on either incipient GWSS populations or, more likely, on a similar native sharpshooter, *H. liturata* Ball, the smoke-tree sharpshooter (Vickerman et al., 2004). *Gonatocerus morilli* is most likely native to the southwest USA. It is anticipated that genetic variability of native *G. ashmeadi* and *G. morrilli* populations will be increased through the release of new stock and this, in turn, could lead to improved biological control. Since initial releases began, over 90 separate recoveries of egg masses parasitized by *G. triguttatus* or *G. fasciatus* have been made in 23 sites over seven counties. This is promising evidence that populations of introduced biological control agents are becoming established in southern California following liberation from quarantine. Biological control of GWSS is seen as a long-term control strategy for suppression of GWSS populations in areas where GWSS has already become established. To this end, the CDFA has established two facilities based in Riverside and Kern counties to mass-produce natural enemies for release, and to monitor populations and spread of these biological control agents post-release.

DETERMINING THE SAFETY OF GWSS PARASITOIDS: NON-TARGET IMPACT STUDIES

Biological control has come under increased scrutiny because there is some evidence that under certain circumstances natural enemies released for the control of a pest species may attack non-target species and adversely affect the populations of these organisms (Hoddle, 2004b). To mitigate unwanted environmental effects that may be associated with exotic natural enemies released for the control of GWSS, native California sharpshooters, such as *Graphocephala atropunctata* (Signoret) and *Xyphon fulgida* (Nottingham), have been studied to see if they are vulnerable to attack by parasitoids native to the southeastern USA and northeastern Mexico (i.e., *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus*) and Argentina (*G. tuberculifemur* (Ogloblin) and undescribed *Gonatocerus* sp.).

All species of GWSS biological control agents have been screened for their ability to parasitize closely related non-target species of Homoptera. These include the southeastern species *H. insolita* (Walker) and the southwestern species *H. liturata* (both proconiine sharpshooters), three sharpshooters of the cicadellini tribe, *Colladonus montanus* (Van Duzee) (Cherry mountain leafhopper), *G. atropunctata* (blue-green sharpshooter) and *X. fulgida* (Red-headed sharpshooter), and other species of leafhoppers from a different subfamily (Cicadellidae: Deltocephalinae) *Euscelidius variegatus* (Kirschbaum), and *Macrosteles fascifrons* (Stål) (Aster leafhopper). To date, the only non-target species susceptible to the agents introduced is *H. liturata*, a species that is implicated in *X. fastidiosa* transmission in agriculture, but exists primarily in desert habitats where mymarid parasitism is not as common as in coastal areas (Al-Wahaibi 2004).

Preliminary observations dealing with native Californian sharpshooters have revealed that GWSS parasitoids may impact their populations. *H. liturata*, from the same tribe and genus and most similar to the GWSS in its egg laying and generalist plant feeding habits and the three *Gonatocerus* species are expected to utilize this native sharpshooter as a host where GWSS and *H. liturata* co-occur. The habitats occupied by three other predominant native sharpshooters, however, have less overlap with GWSS in addition to being from a different taxonomic tribe with different egg laying habits. The habitats of the cicadellini sharpshooters *X. fulgida*, and the green sharpshooter, *Draeculocephala minerva* Ball, both consist of grasses such as Bermuda and Johnson grass, *Cynodon dactylon* (L.) and *Sorghum halapense* (L.) respectively. The blue–green sharpshooter, *Graphocephala atropunctata* (Signoret) (Cicadellini), prefers partially shaded, densely vegetated humid or riparian habitats consisting of trees, native grapes, and weeds such as mugwort and stinging nettle. These unlikely foraging areas combined with the differing taxonomic placement of the non-target sharpshooters, different egg laying habits, and the absence of any records indicating *Gonatocerus* emergence from individual eggs laid within stems make these sharpshooters unlikely alternate hosts for the three *Gonatocerus* parasitoids. Additionally, sticky cards and malaise trap sampling in the southern Californian habitat regions occupied by *G. atropunctata* have yielded no capture of the widespread and established parasitoid *G. ashmeadi* (Boyd et al., unpublished data). Oviposition biology and preferences may exclude some native sharpshooters from parasitization by exotic GWSS parasitoids. For example, on wild grape, *Vitis californica* Benth, *G. atropunctata* oviposition is limited to the terminal 25cm of stem of grape canes and to the newly developing tendrils that develop along the length of the entire grape cane. Furthermore, BGSS eggs are approximately one-half the size of GWSS eggs, are laid singly, and embedded into stems rather than in groups just below the epidermal layer on leaves as is characteristic of GWSS egg masses (Boyd *et al*., this issue). These oviposition characteristics, coupled with the different taxonomic placement, make the BGSS an unlikely host for any of the imported GWSS parasitoids. Additional laboratory testing is currently underway to fully explore any possible non-target effects associated with these parasitoids on the native sharpshooters of California.

GWSS AND NATURAL ENEMY PHENOLOGY IN SOUTHERN CALIFORNIA

Phenological data on GWSS and parasitoid populations (*G. ashmeadi*, *G. fasciatus* and *G. triguttatus*) have been collected for two full years in southern California. In southern California GWSS exhibits two distinct population peaks, the first occurring in spring during which an average of 12 % of GWSS eggs were parasitized and the second in summer, with an average of 19 % of eggs successfully parasitized. This summer figure contrasts with reported parasitism rates of up to 100 % parasitism in some regions (Triapitsyn and Phillips, 2000). Possible explanations of this discrepancy in the parasitism rates is this kind of data being collected in “snapshots” from any given season and from different habitats. The data collected from citrus grown at Ag. Ops at the University of California, Riverside often reflects near to 100 % parasitism rates in individual sampling events but this level of oviposition is generally associated with low GWSS egg numbers and is not reflected in the overall mean estimate of parasitism for any one season. Of the egg masses discovered by *Gonatocerus* spp., 17 % had at least one egg parasitized in spring, compared to 30 % of discovered egg masses utilized at some level in summer, still short of earlier estimates of parasitism rates being around 95-100%.

PARASITOID BIOLOGY

Mymarid parasitoids attacking GWSS eggs are small, approximately 0.5 – 1.5 mm (0.02 – 0.06 inches), and lay their eggs inside GWSS eggs. The developing parasitoids feed on the contents of eggs killing the host. Parasitoid larvae pupate within GWSS eggs and then chew circular holes through the leaf cuticle from which they emerge. *Gonatocerus ashmeadi*, *G. morrilli* and *G. triguttatus* are solitary endoparasitoids that lay one egg into individual GWSS eggs within an egg mass. *Gonatocerus fasciatus* is gregarious, and females deposit more than one egg per GWSS egg yielding multiple parasitoids per host egg (Triapitsyn et al., 2003).

The density of searching females has a significant effect on the sex ratio of progeny produced. When female *Gonatocerus* parasitoids fail to encounter other ovipositing females, progeny output is strongly female biased. Laboratory experiments indicate that approximately 1 males: 8 females, 1:14 and 1:9 are produced for *G. ashmeadi, G. triguttatus* and *G. fasciatus*, respectively. Increasing the number of conspecific ovipositing females from one to two per arena, significantly reduces percentage of female offspring by up to 15.0% for all three *Gonatocerus* species. These results suggest that local mate competition affects progeny production and more males are produced when females encounter conspecifics who are producing daughters with whom their sons may mate.

No-choice laboratory studies have shown that progeny production for *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* was greatest from GWSS eggs 3, 4, and 2 days of age, respectively. Furthermore, each parasitoid species was able to utilize a range of egg ages around their most preferred age, these being eggs 1-4, 3-6, and 1-3 days of age for *G. ashmeadi*, *G. triguttatus* and *G. fasciatus*, respectively. GWSS eggs 8 to 10 days of age produced few parasitoid progeny. This most likely occurred because of the advanced stage of development of the GWSS embryo (Irvin and Hoddle, 2004). However, when given a choice between GWSS eggs 1, 3, and 5 days of age presented simultaneously to *G. ashmeadi* and *G. triguttatus* no oviposition preferences were observed. This suggests that these two parasitoids will attack host eggs without preference as long as eggs are of a suitable age for oviposition. The choice studies indicated *G. fasciatus* had preference for GWSS eggs 1-3 days of age while eggs 5 days of age were not utilized. We suspect the small size of *G. fasciatus* in comparison to *G. ashmeadi* and *G. fasciatus* limits the range of GWSS egg ages available for parasitism. This may occur because the smaller ovipositor of *G. fasciatus* may be unable to pierce the chorion (i.e., egg shell) of older eggs as they harden during maturation and this would prevent successful parasitism of eggs as they develop.

ENHANCING PARASITOID SURVIVAL AND PARASITISM RATES IN THE FIELD

Most agricultural environments are unfavorable habitats for natural enemies because herbicides remove potential shelter and floral resources (Gurr et al., 2003). In the laboratory, it has been demonstrated that honey-water and buckwheat (*Fagopyrum esculentum* Moench) flowers significantly increased longevity of male and female *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* up to 94.6%, 92.4% and 93.1%, respectively, compared with water. These results indicate that resource procurement maybe extremely important for enhancing parasitoid survival in agroecosystems. Increased longevity of female parasitoids resulting from resource procurement may enhance biological control of *H. coagulata* because greater female longevity may increase host encounter and parasitization rates.

Longevity of *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* on citrus flowers and *H. coagulata* excrement was equivalent to that on water. This indicates that these field resources may not supply parasitoids with adequate nutrition to maximize survival. Understory management (i.e., the deliberate management of flowering plants beneath orchards and vineyards) is potentially one way to enhance parasitoid populations in agricultural systems thereby leading to improved pest control by natural enemies (Baggen and Gurr, 1998). Sowing flowering plants [e.g., buckwheat, dill (*Anethum graveolens* L.), or alyssum (*Lobularia maritime* L.)] as an understory in citrus orchards harboring *H. coagulata* could potentially provide pollen and nectar to *Gonatocerus* species. Additionally, excrement from brown softscale, (*Coccus hesperidum* L., a common pest in citrus orchards, increased survival times by up to 85.2% for both sexes of *G. ashmeadi*, *G. triguttatus*, and *G. fasciatus* compared with citrus foliage alone, suggesting that in citrus orchards, low non-damaging *C. hesperidum* populations may also be beneficial for enhancing parasitoid survival and could enhance biological control of *H. coagulata*.

THE INVASIVE POTENTIAL OF GWSS

GWSS has shown strong invasive potential having established outside of its home range in California, French Polynesia, and Hawaii. Modeling that combines regional climate data and relevant biological information has indicated that California's premier wine growing areas of Napa, Sonoma, and Mendocino counties are vulnerable to invasion by GWSS. States north of California, such as Oregon and Washington, also with substantial grape industries, may be too cold to harbor permanent populations of GWSS (Hoddle, 2004a). The major wine-growing regions of New Zealand, Australia, the Bordeaux region of France, most areas of Spain, as well as central and southern Italy have climates conducive to GWSS establishment and proliferation should it be accidentally introduced (Hoddle, 2004a).

Data on GWSS in Tahiti is sobering where this pest has exhibited uncontrolled population growth due to an abundance of suitable host plants (native and exotic), mild climate that permits year round breeding (this is in direct contrast to California where there are just 2 generations [spring and summer] each year), lack of natural enemies, and no obvious competitors in urban or natural settings. Naturally occurring parasitism of GWSS eggs is very low on the island of Mo'orea. Surveys indicated that < 2% of individual eggs were attacked in just 4% of egg masses collected. Of those egg masses attacked, 44% of the eggs in those masses were parasitized (Table 1). The parasitoid responsible for attacking GWSS egg masses is a platygasterid, a family that does not specialize on sharpshooters, but will parasitize various species of leafhoppers. The data on parasitism in Tahiti indicate that there are no specialized parasitoids attacking GWSS, only a few eggs in an egg mass are attacked indicating inefficient and opportunistic exploitation, and only males were reared from GWSS eggs suggesting poor host quality as females did not oviposit fertilized female eggs. Predation on GWSS eggs most likely was a result of spider activity. These data clearly indicate GWSS populations in French Polynesia are in natural enemy free space. A classical biological control initiative against GWSS has been launched and is a cooperative enterprise between the University of California at Riverside and Berkeley, and the French Polynesian Government.

Table 1
Data on GWSS egg mass survivorship collected over the period September 3-9 2003 on the Island of Mo'orea in French Polynesia.

No. of GWSS Eggs Examined	No. Eggs GWSS Parasitized	No. GWSS Eggs Eaten	No. GWSS Died of Natural Causes	No. GWSS Eggs that Nymphs Emerged From
2586 (246 egg masses examined)	32	444	50	2060
% of eggs	1.24% (4% of collected egg masses were attacked)	17.17%	1.93%	80%

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