

SEASONAL POPULATION DYNAMICS OF GLASSY-WINGED SHARPSHOOTER EGG PARASITIDS: VARIABILITY ACROSS SITES AND HOST PLANTS

Project Leader:

Joseph G. Morse
Department of Entomology
University of California
Riverside, CA 92521

Cooperators:

David J.W. Morgan
Calif. Dept. Food & Agric.
Mount Rubidoux Field Station
Riverside, CA 92501

Jonathan M. Lytle
Department of Entomology
University of California
Riverside, CA 92521

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ABSTRACT

The California Department of Food & Agriculture (CDFA) has a number of sites in southern California where they are releasing egg parasitoids of the glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*; formerly *H. coagulata*). To date, species released include *Gonatocerus ashmeadi*, *G. fasciatus*, *G. walkerjonesi*, and *G. triguttatus*. Very recently, two new species, *G. morrilli* (first released at our sites 5/2/06) and a strain of *Anagrus epos* from Minnesota (first released at our sites 5/17/06) have been released. We recently recovered *G. morrilli* (on 8/22/06) but not yet *Anagrus epos* (*A. epos*). CDFA monitors for parasitoid establishment and population dynamics at release sites. This project is intended to complement and expand the scope of this monitoring with an eye towards improving our understanding of the benefit of releasing alternative parasitoid species and how well they are surviving, dispersing, and impacting GWSS populations in southern California.

INTRODUCTION

One of CDFA's parasitoid release sites in southern California is Field 7H on the University of California, Riverside (UC Riverside) campus. A two-year field study in and around this release site was conducted to examine the temporal and host plant distribution of *Homalodisca* oviposition and associated egg parasitism (Al-Wahaibi 2004). In the current project, we plan to expand on this study and monitoring done by CDFA in an attempt to improve our understanding of the population dynamics of endemic and released parasitoids in and around release sites. Although control programs appear to be effective at reducing glassy-winged sharpshooter (GWSS) populations, biological control is a more sustainable and environmentally friendly means of contributing to vector reduction and may have to suffice in much of California where chemical control is either impractical (e.g., urban areas) or economically unfeasible.

In the two-year (July 2001 – June 2003) study by Al-Wahaibi (2004) around CDFA's release site on the UC Riverside campus, parasitism was due to a total of eight parasitoid species with *Gonatocerus ashmeadi*, *Ufens principalis* (previously *Ufens* A, Al-Wahaibi et al. 2005), *Ufens ceratus* (previously *Ufens* B), and *G. walkerjonesi* being the most abundant. *Ufens* spp. were dominant on jojoba while on other plants, *Gonatocerus* species tended to dominate. Across all ten host plants sampled, ranked percent parasitism was *G. ashmeadi* (27.4%), *U. principalis* (19.8%), *U. ceratus* (2.9%), *G. walkerjonesi* (2.1%), *G. incomptus* (0.4%), *G. novifasciatus* (0.3%), *G. triguttatus* (0.1%), and *G. fasciatus* (0.01%). Note, however, that these data may have been biased by the proximity of nearby hosts harboring smoke-tree sharpshooter and high levels of *Ufens* spp. on jojoba.

The taxonomy of the "*G. morrilli*" complex was revised when it was discovered that what had been called *G. morrilli* was actually 2 species that were difficult to tell apart. These are the newly names *G. walkerjonesi* which is either native to California or was unintentionally introduced from some other area (similar to species from Central America) and *G. morrilli* which is native to Florida, Texas, and northeastern Mexico. In the past, CDFA released what they were calling "*G. morrilli*" but when genetic methods became available to distinguish the two species, it was realized that the culture was actually *G. walkerjonesi*. At that time, *G. morrilli* was recollected from Texas, a colony was started in California, and releases have been made starting 6/6/06 at four of our release sites.

Another new listing in our recovery data is the newly named *G. morgani*. This species is likely native to California or was unintentionally introduced into California. It was overlooked in the past, perhaps because it was not common on smoke-tree sharpshooter and was thus missed prior to GWSS's establishment and proliferation in California.

Anagrus epos was collected in Minnesota by Dr. Roman Rakitov (Center for Biodiversity, Illinois Natural History Survey, Champaign, Illinois) near Glyndon, Clay Co., Minnesota, from egg masses of *Cuerna fenestella* Hamilton (a native, univoltine proconiine sharpshooter) on *Solidago* sp. (goldenrod, *Compositae*) and *Zigadenus* sp. (death camus, *Liliaceae*) and sent to Dr. Serguei Triapitsyn at the UC Riverside quarantine facility under an appropriate permit (Hoddle & Triapitsyn 2004, Triapitsyn & Rakitov 2005). A permit for release from quarantine was obtained in 2005 by Dr. David Morgan and this strain is presently being reared by CDFA and has already been released at a few field sites in California.

OBJECTIVES (As Modified)

Monitor GWSS egg parasitoids in six areas in southern California (three coastal, three interior sites) on citrus within CDFA's parasitoid release sites. Focus on evaluation of two new species, i.e. *A. epos* and *G. morrilli*.

The type of monitoring data we collect at each site is listed below. We are using CDFA's basic monitoring protocol with modifications. Note that we have three replicated sampling plots at our Mission Viejo and San Juan Capistrano sites, two at UC Riverside Field 7H, only one at Irvine (because the site is too small for two), and six at Temecula. For 2006, we are releasing and sampling in one sampling plot per site in order to maximize chances of detecting establishment of newly released parasitoids.

1. Sticky traps to monitor for adult GWSS levels: Use 10 yellow sticky traps in each plot to assess adult GWSS activity levels every two weeks.
2. Leaf sampling: Count and collect the number of fresh GWSS egg masses on 10 leaves collected from the end of branches on each of 10 trees in each plot every two weeks. In contrast to method three, this is intended to return a less-biased estimate of GWSS egg mass levels. Old egg masses are counted, but not collected. The egg mass sampling is mainly intended to estimate recent GWSS egg mass levels and to serve as a means of collecting egg masses for parasitoid rearing.
3. Time search for GWSS egg masses: Do six two-minute time searches near the center of each plot every two weeks, looking for, counting, and collecting viable (new) GWSS egg masses. Continue sampling an additional 30 minutes until a minimum of five egg masses are found from methods two and three combined.
4. Parasitoid emergence data: Using egg masses collected in methods two and three (aim for 5-10 egg masses per date if possible), return egg masses to the lab and rear out and identify parasitoid species that are present.

RESULTS

Based on discussions with our CDFA cooperators, we have made several changes in project objectives, experimental design, and methodologies because of low levels of GWSS at several initial monitoring sites, changes in the species / strains of parasitoids CDFA has reared and released, the number of parasitoids they have been able to produce over this past year (this has been a very difficult year as far as rearing GWSS egg masses which are the cornerstone of the rearing program), and what makes practical sense within an applied management program (Shea et al. 2002) given advances in our knowledge regarding *Gonatocerus* species and the new strain of *A. epos* from Minnesota (see below).

To briefly summarize our research activity to date, we have monitored parasitoid activity at a total of 13 sites in southern California. Three sites were dropped because GWSS and parasitoid activity were too low (Mecca 1 and 2, BC = UC Riverside Biological Control grove), two were dropped when the grove was sold and the grower turned off the water for over a month resulting in about one-half of the trees dying (Temecula 2 and 3), two were dropped because the organic grower did not control weeds and let the Argentine ant population get completely out of control resulting in a crash in GWSS egg mass levels (Temecula 1 and 4), and one was dropped when we decided to switch to lemon blocks at all sites (Crafton Hills was navel orange). At present, we have six sampling / parasitoid release sites, three in the coastal area (Irvine, Mission Viejo, San Juan Capistrano) and three in the interior area (Corona, UC Riverside Field 7H, and Temecula).

2005 Parasitoid data

In 2005, we made a total of 98 collections from 13 different field sites. Out of a total of 2,647 parasitoids recovered, 61.9% (1639) were *G. walkerjonesi*, 29.5% (782) *G. ashmeadi*, 4.5% (120) *Ufens* spp. (either *U. principalis* or *U. ceratus*), 2.5% (66) *G. novifasciatus*, 1.2% *G. sp.* (32) (identity could not be determined due to specimen condition), and 0.3% (8) were *G. triguttatus*. This latter species was collected only once at a single site.

2006 Parasitoid data

A total of 3,610 *G. morrilli* have been released in 2006 at four of our release sites (UC Riverside Agricultural Operations, Irvine, Mission Viejo, and San Juan Capistrano). Due to limitations in how many of these parasitoids can be reared for release, we have not yet released this species at the Corona or Temecula sites.

A. epos has proven difficult for CDFA to rear and we have also experienced problems rearing this species in one of the two colonies at UC Riverside (it is doing very well on the second floor insectary room but not at all well on the third floor quarantine room). Additional work is needed to determine why this species is difficult to rear in some cases but not others. To date, we have made only a single release of *A. epos* at each of two release sites (180 wasps on 5/17/06 at Agricultural Operations, 300 wasps on 5/25/06 at Irvine).

We are only part way through our 2006 survey at the six parasitoid release sites (parasitoids are still being reared out from egg masses collected in early September) but so far we have recovered 595 parasitoids in total. Within the three interior sites, our best site by far is Agricultural Operations and both the Corona and Temecula sites are yielding few GWSS egg masses with minimal parasitoid diversity (we have recovered only *G. ashmeadi* at these two sites). A key finding is a single

G. morrilli recovered at Agricultural Operations on 8/22/06, only 35 days after it was first released at this site on July 18, 2006.

Among the three coastal sites, all three sites started out quite strong (good GWSS egg mass and parasitoid recovery) but Argentine ants have taken over at the Irvine site despite our instituting a very aggressive ant baiting program. This site is an organic lemon grove and thus we were restricted to using boric acid bait stations as the only organically approved treatment. A bait station was placed under each of 31 sample trees and despite baiting continuously for 16 weeks so far, we have not caused a significant reduction in Argentine ant levels (compared to the level of ants in 16 untreated control trees at the end of the block). Argentine ants feed on GWSS egg masses and disturb nymphs and adults. GWSS egg mass levels have dropped at this site and because non-organic treatments cannot be used (e.g., a very effective chlorpyrifos ground spray), we may have to abandon this site.

The coastal parasitoid data is an interesting contrast to data from the interior region. *G. ashmeadi* (only 5.7% of 350 parasitoids recovered so far) is present on the coast but the most common species are *G. walkerjonesi* (67.4%) and *G. morgani* (25.1%). We have also seen low numbers of *G. novifasciatus* (6.0%) and *Ufens* spp. (1.7%) on the coast.

CONCLUSIONS

Despite 2005 releases of *G. fasciatus* and *G. triguttatus*, limited numbers of these species have been recovered. The latter species is still produced and released as some success in recoveries has occurred in past years. The CDFA facility in Riverside has ceased producing and releasing *G. fasciatus* as no recoveries have been recorded in the past two years. We find it interesting that we are recovering so many *G. walkerjonesi* in the coastal region whereas a common impression held by many is that *G. ashmeadi* predominates in California. We believe this is because many biological control researchers have worked in the Riverside and other interior areas but relatively few have studied GWSS egg parasitoids in coastal regions. It is not surprising that species levels vary with geographic region and climate. We are very encouraged by recovering *G. morrilli* from field samples only 35 days after it was first released and believe it is much too early to be discouraged by our not yet recovering *Anagrus epos*. It will be important to continue these studies an additional year once greater numbers of *A. epos* have been released in the field. In particular, we are hoping *A. epos* may show up early in the year on first generation eggs of GWSS when *Gonatocerus* egg mass parasitism is generally quite low.

As a consequence of these and similar studies undertaken by the CDFA, a greater investment is being made toward the production, release, and monitoring of *G. morrilli*. This species is currently the second most produced biological control agent by the CDFA in both of its production facilities and it is being released over a range of environments including urban, organic, coastal, and inland locations.

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THE *ANAGRUS EPOS* COMPLEX: A LIKELY SOURCE OF EFFECTIVE CLASSICAL BIOLOGICAL AGENTS FOR GLASSY-WINGED SHARPSHOOTER CONTROL

Project Leaders:

Joseph G. Morse
Department of Entomology
University of California
Riverside, CA 92521

Richard Stouthamer
Department of Entomology
University of California
Riverside, CA 92521

Cooperators:

Serguei V. Triapitsyn
Department of Entomology
University of California
Riverside, CA 92521

David J. W. Morgan
CDFA
Mount Rubidoux Field Station
Riverside, CA 92501

Jonathan M. Lytle
Department of Entomology
University of California
Riverside, CA 92501

Rodrigo Krugner
Department of Entomology
University of California
Riverside, CA 92501

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ABSTRACT

The purpose of the work planned in this project is to determine whether the “Minnesota strain” of the mymarid, *Anagrus epos* Girault, we have in culture on the glassy-winged sharpshooter (GWSS) is the same species as *A. epos* strains previously released in California, how it compares with other “*A. epos*” strains, and whether there are other strains of “*A. epos*” that should be imported for biological control of GWSS. Without understanding what species we have and how the Minnesota strain is related to similar strains, it is difficult to know how to proceed in selecting strains of this species to culture for mass-rearing and release in California for GWSS control. Concurrently, we will evaluate field releases and establishment of the Minnesota *A. epos* strain at release sites in southern California.

INTRODUCTION

Anagrus epos is a common and seemingly widespread egg parasitoid of leafhoppers (Cicadellidae) in North America. It was first described from a collection in Illinois in 1911 (Girault 1911). Location records for this species also include Colorado, Kentucky, New Mexico, and New York in the U. S. as well as Baja California and Sonora in Mexico (Triapitsyn 1998). While commonly collected as a parasitoid of grape leafhopper species (*Erythroneura* spp.), a recent collection of *A. epos* from the egg mass of the sharpshooter genus *Cuerna* in Minnesota was the first time this species had been collected from a sharpshooter species (Hoddle & Triapitsyn 2004). Wasps from this collection have been reared continuously since June 2004 in the University of California Riverside (UCR) Quarantine facility on eggs of the glassy-winged sharpshooter (GWSS). This species is particularly promising for application in the biological control of GWSS because it is a gregarious species and fourteen or more wasps emerge from each egg. Another apparent advantage of this species is that it will also parasitize the eggs of several other leafhopper species (R. Krugner, unpublished data), thus allowing it to expand its numbers even at times of the year when GWSS eggs are not present. We also expect this strain may do quite well in the colder regions of central and northern California based on where it was collected.

Like many minute parasitoids, identification to species in this group is exceedingly difficult because of the lack of adult morphological features. Species identifications have been made using light microscopy to determine the presence of key morphological features for *A. epos*. A recent taxonomic revision of the genus *Anagrus* associated with vineyards in North America (Triapitsyn 1998) has shown that: 1) more species are present than previously thought; 2) some species have a very wide geographic distribution; and 3) relatively few morphological characters are available for distinguishing these species, leaving several authors to think that *A. epos* is not a single species but a complex of different species (e.g., Pickett et al. 1987). The morphological characters that are used for differentiating closely related *Anagrus* spp. can be variable and thus, species limits are often difficult to assess without supporting data from their biology and from DNA sequences. Due to limitations on what is practical (economically) to rear and mass-release and also because of restrictions on importing and releasing exotic parasitoids in California without understanding their taxonomy, we feel we must better understand this species complex. We intend to use three approaches to determine the species identity of different *A. epos* populations: (1) reassess key morphological features using scanning electron microscopy (SEM) to determine if subtle morphological differences exist between *A. epos* populations which could indicate species differences (Dr. Triapitsyn will conduct this work); (2) conduct mating compatibility studies to determine if different populations of *A. epos* are reproductively isolated, or if mating occurs, whether offspring from different strains are viable, thereby defining species groups on the basis of successful interbreeding (Ph.D. student John Lytle working with Dr. Morse); (3) determine if molecular differences exist between *A. epos* populations collected from different regions by comparing mitochondrial and ribosomal DNA sequences