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

Project Leaders:
Joseph G. Morse and Richard Stouthamer
Department of Entomology
University of California
Riverside, CA 92521

Cooperators:
Serguei Triapitsyn
Department of Entomology
University of California
Riverside, CA 92521

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

Rodney Mendes
CDFA, PDCP
Riverside, CA 92507

Jonathan M. Lytle and Nick C. Toscano
Department of Entomology
University of California
Riverside, CA 92521

Reporting Period: The results reported here are from work conducted May 2005 to September 2005.

ABSTRACT
The purpose of the work planned in this project is to determine whether the "Minnesota strain" of the mymarid, *Anagrus epos* Girault (*A. epos*), we have in culture on 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 six 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 UC Riverside quarantine facility on eggs of the GWSS. This species is particularly promising for application in the biological control of the GWSS because it is a gregarious species and twelve 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. The purpose of the work proposed here is to determine whether the "Minnesota strain" of "*A. epos"* we have in culture on GWSS is actually this species, how it compares to 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 it is related to other 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 to control GWSS. 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 *Anagrus epos* populations: (1) Reassess key morphological features using scanning electron microscopy (SEM) to determine if subtle morphological differences exist between *Anagrus 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 *Anagrus epos* populations collected from different regions by comparing mitochondrial and ribosomal DNA sequences (Dr. Stouthamer). Molecular dissimilarities generally indicate the existence of different species. Results from these three methods of investigation (morphology, behavior, and genetics) will be evaluated together to establish the identity of the species in the *A. epos* complex. Once the different species have been determined, we will test them for their suitability in the biological control of GWSS using laboratory studies and field release evaluations (Drs. Morgan and Morse).

OBJECTIVES

1. Examination of male and female *A. epos* complex populations for unique morphological characters
2. Molecular characterization of mitochondrial and ribosomal DNA *of A. epos* populations
3. Mating compatibility studies between *A. epos* strains
4. Field release and evaluation of the "Minnesota strain" of *A. epos*

RESULTS

Progress on Objectives 1-3

The experimental plan laid out in our research proposal was to collect dead specimens of various *A. epos* strains and related species for taxonomic examination (Objective 1) and genetic work (Objective 2) in Year 1. Objective 3 is scheduled for Year 2 once we have the results of Objectives 1 and 2 research, which will tell us which strains of *A. epos* to concentrate on other than the "Minnesota strain" we currently have in culture on GWSS egg masses.

The mymarid *Anagrus epos* Girault was collected and reared in early June 2004 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 UCR quarantine facility under an appropriate permit (Hoddle & Triapitsyn 2004, Triapitsyn and Rakitov 2005). This is the first representative of the genus *Anagrus* ever reared from eggs of a proconiine sharpshooter. At the UCR quarantine laboratory during summer 2004, S. Triapitsyn and V. Berezovskiy were able to establish a colony of this species on eggs of GWSS, which is a fictitious host for *A. epos* (GWSS does not occur in Minnesota). *Anagrus epos* is a gregarious species: 3-5 adult wasps emerged from smaller eggs of the original host, *Cuerna fenestella*, whereas up to 12 adult wasps emerged from larger eggs of GWSS. Under quarantine laboratory conditions (temperature 24°C, RH ca. 50%), the first two generations of *A. epos* developed from egg to adult within 20-21 days; for unknown reasons, it took the next two generations much longer (more than 30 days) to develop under the same conditions. In September 2004, the colony of *A. epos* was turned over to Dr. Joseph Morse, and it has been successfully maintained since then by Rodrigo Krugner, a Ph.D. graduate student. A release permit was received by Dr. David Morgan (CDFA), who established another colony of *A. epos* at the Mt. Rubidoux CDFA rearing facility in Riverside and has released this species in selected locations in California against GWSS (http://www.cdfa.ca.gov/phpps/pdcp/BioCtrlRep/gwBioIndex.htm).

Triapitsyn (1998) re-described *A. epos* from the type material and other specimens collected in Centralia, IL, and also indicated its additional distribution in North America (Mexico: Baja California, Sonora; USA: Colorado, Illinois Kentucky, New Mexico). In CO and NM, it is a parasitoid of *Erythroneura* leafhoppers on grapes; also indicating that morphologically, it is a variable species (and thus possibly a complex of several cryptic species). The specimens from Minnesota are within this variation range and are possibly also members of such a complex. The species related to *A. epos* are *Anagrus daanei* S. Triapitsyn (Canada: British Columbia; USA: California, Michigan, New York, Washington) and *Anagrus tretiakovae* S. Triapitsyn (Mexico: Baja California, Coahuila; USA: Arizona, Delaware, Illinois, Michigan, Maryland, New Mexico, New York, Washington); in AZ and NM (and Mexico), it is a known parasitoid of *Erythroneura* leafhoppers on grapes.

For the planned molecular and morphological comparison, S. Triapitsyn made several attempts to collect *A. epos* and *A. tretiakovae* during summer 2005 but they were not as productive as expected for the following reasons. First, the USDA importation permit to bring *Anagrus* spp. into UCR quarantine was received only September 9, 2005, after a long delay. Thus, the most productive method of collecting specimens (collecting large amounts of plant material showing signs of leafhopper damage, sending it to the UCR quarantine facility, and rearing it out there) could not be utilized. Second, the primary habitat of *A. tretiakovae* in Arizona (the organic table grape vineyards near Dateland, in the Harquahala Valley, and near Stanfield, AZ) were completely removed for economic reasons (per telephone conversation with Steve Pavich, owner). At S. Triapitsyn's request, Doug Yanega, the Senior Museum Scientist at the UC Riverside Entomology Research Museum, attempted to collect *A. tretiakovae* in the wine vineyards near Tucson, AZ, but neither the parasitoid nor its hosts, *Erythroneura* spp., were found there.

Third, our collaborator's attempts to rear *A. epos* from grapes at Caborca and Costa de Hermosillo, Sonora, Mexico, were also unsuccessful this summer. Agustín Fú-Castillo notified us that all the vineyards in the vicinity of Sonora were treated with an insecticide against the vine mealybug, and as a result, the usually very common *A. epos* could not be collected this summer.

Fourth, S. Triapitsyn made a trip to Grand Junction/Palisade area of Colorado in mid July 2005 to try to collect *A. epos* in the vineyards there. He found very light leafhopper infestations of *Erythroneura vulnerata* Fitch in the vineyards in Palisade, but they were present only in the untreated rows where a weather station was located. Unfortunately, no *Anagrus* emerged from numerous leaves with signs of leafhopper damage (from inside the vine) he collected. An *Anagrus* sp., collected by sweeping grape leaves infested with *E. vulnerata,* unfortunately turned out to belong to an unrelated species, *A. nigriventris* Girault.

Anagrus erythroneurae S. Triapitsyn & Chiapinni (it will be used as an out-group for comparison) is presently being collected (September 2005) in Fresno-Parlier area by our collaborators Dr. Kent Daane and Mr. Glenn Yokota (UC Berkeley). Later in the fall or next spring, they will also assist by collecting *A. daanei* from blackberry and/or grapes.

Finally, another attempt to collect *A. epos* and perhaps *A. tretiakovae* was made by S. Triapitsyn September 26-27, 2005. He was able to collect four males of *A. tretiakovae* by sweeping a pesticide-free vineyard and preserved them in 95% ethanol for molecular study. In addition, he collected leaves with leafhopper damage and shipped them under a permit to the UCR quarantine facility. We are hopeful that additional specimens will emerge.

Progress on Objective 4

As laid out in the research proposal funding this work, we have initiated monitoring of endemic and released parasitoids of GWSS at each of six Eureka lemon field sites in southern California (for details see the progress report in this Proceedings by Morse). A grant from the UC Pierce's Disease Research Program funded Years 1 (2004-05) and 2 (2005-06) of field monitoring whereas Year 2 (2006-07) will be funded by this (the *Anagrus*) project.

We are on track to study the release of the Minnesota strain of *A. epos* at each of the six study sites commencing with releases in February 2006.

Progress on Related Objectives

Ph.D. student Rodrigo Krugner has been rearing and studying the Minnesota strain of *A. epos*, first in quarantine and more recently, in the UCR Insectary after Dr. Morgan received the permit allowing it to be taken out of quarantine. Mr. Krugner's research is focusing on the basic biology of *A. epos* including (1) host specificity studies, (2) host egg age preference, (3) longevity of *A. epos* adults, (4) fecundity and fertility, (5) development of a temperature-dependent (degree day) model of the immature stage, and (6) sex allocation by *A. epos* females.

CONCLUSIONS

We are slightly behind schedule in collecting specimens of various *A. epos* strains and related species for taxonomic examination and genetic work but have done what is possible given the poor luck we've had this year in field collecting these parasitoids. However, we are much further along than would have been predicted in biological studies with *Anagrus epos* and have made a breakthrough in rearing this strain, which should allow substantial progress in field research over 2006-07.

REFERENCES

Girault, A. A. 1911. Descriptions of North American Mymaridae with synonymic notes on described genera and species. Trans. Amer. Entomol. Soc. 37: 253-324.

Hoddle, M. S., and S. V. Triapitsyn. 2004. Searching for and collecting egg parasitoids of glassy-winged sharpshooter in the central and eastern USA, pp. 342-344. *In*: Proceedings of the 2004 Pierce's Disease Research Symposium, December 7-10, 2004, Coronado Island Marriott Resort, Coronado, California, organized by California Department of Food and Agriculture (compiled by M. Athar Tariq, S. Oswalt, P. Blincoe, A. Ba, T. Lorick and T. Esser), Copeland Printing, Sacramento, California.

Pickett, C. H., L. T. Wilson, D. González, and D. L. Flaherty. 1987. Biological control of variegated grape leafhopper. Calif. Agric. 41 : 14-16.

Triapitsyn, S. V. 1998. *Anagrus* (Hymenoptera: Mymaridae) egg parasitoids of *Erythroneura* spp. and other leafhoppers (Homoptera: Cicadellidae) in North American vineyards and orchards: a taxonomic review. Trans. Amer. Entomol. Soc. 124: 77-11.

Triapitsyn, S. V., and R. A. Rakitov. 2005. Egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of *Cuerna* sharpshooters (Hemiptera: Cicadellidae) in the USA. Abstracts, 12th International Auchenorrhyncha Congress and 6th International Workshop on Leafhoppers and Planthoppers of Economic Significance, University of California, Berkeley, 8-12 August 2005.

FUNDING AGENCIES

Funding for this project was provided by the University of California's Pierce's Disease Grant Program.