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

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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advantage of this species is that it will also parasitize the eggs of several other leafhopper species (R. Krugner, unpublished data), thus allowing it to expand its numbers even at times of the year when GWSS eggs are not present.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

(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 (Dr. Morgan and Dr. 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

Our revised experimental design is to collect dead specimens of various *A. epos* strains and related species for taxonomic examination (Objective 1) and genetic work (Objective 2) in years 1-2. Objective 3 is scheduled for year 3 once we have the results of Objective 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.

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 closely 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), *A. tretiakovae* is a known parasitoid of *Erythroneura* leafhoppers on grapes.

Collection Material

See our 2005 progress report (Morse & Stouthamer 2005) for a listing of *Anagrus* spp. collections made in summer 2005. Additionally, during fall 2005, we obtained large numbers of *A. daanei* from Fresno Co. (courtesy of K. Daane and G. Yokota, UC KAC) and *A. tretiakovae* (reared at UCR quarantine from eggs of *Erythroneura* spp. on grape leaves, collected in Albuquerque, New Mexico by S. Triapitsyn). In 2006, the following collections were made: *A. daanei* from Washington (courtesy of L. Wright, Washington State University, Prosser), *A. erythroneurae* Trjapitzin & Chiappini from Oasis and Temecula, California (reared from eggs of *Erythroneura variabilis* Beamer on grapes), and *A. epos* from Grand Junction and Palisade, Colorado (reared by S. Triapitsyn from eggs of *Erythroneura vulnerata* Fitch on grapes and also from eggs of *E. ziczac* Walsh on Virginia creeper). Thus, all necessary collections for this study have been made, with the exception of *A. epos* from Sonora, Mexico, where all the grapes were treated with insecticides against the vine mealybug, resulting in elimination of *Erythroneura variabilis* leafhoppers there. Luckily, there are enough preserved *A. epos* vouchers stored in a freezer at the UCR Entomology Research Museum, reared by S. Triapitsyn in 1994 in Sonora from eggs of *E. variabilis* on grapes. These were successfully sequenced in Dr. Stouthamer's lab, and also were used for morphological studies.

Morphological Studies

Scanning electron micrographs (SEMs) of the antennae and bodies were taken for the following specimens: *A. epos* (Grand Junction, Colorado), *A. epos* (Sonora, Mexico), and *A. epos* (Minnesota origin). Digital photographs (using the Automontage system) of the antennae, forewings, and bodies were taken for the following specimens: *A. epos* (Grand Junction, Colorado), *A. epos* (Sonora, Mexico), *A. epos* (Minnesota origin), and *A. epos* (Illinois). Certain body part measurements were taken from the following specimens: *A. epos* (Grand Junction, Colorado), *A. epos* (Sonora, Mexico), *A. epos* (Minnesota, both original and CA progeny), and *A. epos* (Illinois). Morphometric studies of these specimens are now underway and should be completed shortly.

Specimens and DNA Preparation

Nine *Anagrus* species were obtained from 15 collection sites for molecular identification (Table 1). Two individuals from each population were chosen to prepare template DNA by using 5% Chelex-TE solution. Each individual was ground in 45 ul 5% Chelex solution and then five ul of proteinase K were added in a 0.6 ml centrifuge tube. The mix was incubated at 55°C for one hr and 99°C for 10 min.

PCR Methods and Results

PCR was performed to yield the 28sD2 (ribosomal cistron) and CO1 (mitochondrial gene) regions with template DNA. Reaction conditions for the 28sD2 region were three min at 94°C, followed by 30 cycles of 45 sec at 94°C, 30 sec at 55°C, 90 sec at 72°C, and a final extension for three min at 72°C. Reaction conditions for the CO1 region were three min at 94°C, followed by 34 cycles of 45 sec at 94°C, 30 sec at 43.5°C, 90 sec at 72°C, and a final extension for five min at 72°C. We

were unable to obtain the CO1 region from the species. Therefore only the 28sD2 region was further treated. Specimens showing weak or no bands were excluded from sequencing. Thus, one PCR amplicon per group was sequenced.

The species of the genus *Anagrus* are very small and lack easy morphological characters that can be used for identification. The D2 sequence has been shown to be a sequence that is quite conserved within a species but is different between species. The work here illustrates the preponderance of species that would morphologically be classified as *A. epos*, but are different species (new species one, two, and three, each different from the MN "*A. epos"* and each other) whereas species four morphologically resembles *A. daanei*.

Table 1. *Anagrus* species used for molecular identification.

Collection	Genus	Species	Collection site
1	*Anagrus*	*epos*	UCR culture, originally collected near Glyndon, Clay Co., MN, 2004
2	*Anagrus*	*nr epos new species 1*	Campo Experimental INIFAP, Sonora, Mexico, 1994
3	*Anagrus*	*nr epos new species 1*	Near Caborca, Sonora, Mexico, 1994
4	*Anagrus*	*nigriventris*	UCR, Riverside, Riverside Co., CA, 2004
5	*Anagrus*	*daanei*	Kingsburg, Fresno Co., CA, 2005
6	*Anagrus*	*erythroneurae*	WSU-Prosser Research Center, Prosser, Benton Co., WA, 2005
7	*Anagrus*	*erythroneurae*	Oasis, Coachella Valley, Riverside Co., CA, 1994
Rearing in progress	*Anagrus*	*erythroneurae*	Temecula, Riverside Co., CA, 2006
8-10, 12	*Anagrus*	*tretiakovae*	Albuquerque, Bernalillo Co., NM, 2005
11	*Anagrus*	*tretiakovae*	Pavich vineyard, Harquahala Valley, Maricopa Co., AZ, 1994
13	*Anagrus*	*nr epos new species 2*	Grand Junction, Mesa Co., CO, 2006 (ex. *Erythroneura vulnerata* eggs)
14	*Anagrus*	*nr epos new species 3*	Palisade, Mesa Co., CO, 2006 (ex. *Erythroneura ziczac* eggs)
15	*Anagrus*	*nr daanei new species 4*	WSU-Prosser Research Center, Prosser, Benton Co., WA, 2006

Progress on Objective 4

We have initiated monitoring of endemic and released parasitoids of GWSS at each of six field sites in southern California (for details see the progress report in this Proceedings by Morse, Morgan, and Lytle). CDFA has had trouble rearing *A. epos*, so far we have made only two releases, of 300 and 180 *A. epos,* respectively at a single coastal and interior site using wasps from the UCR's colony (5/17/06 interior; 5/25/06 coastal), but we hope that CDFA will be able to produce wasps that can be released at additional sites in October 2006. So far we have not recovered *A. epos* from the two release sites but we feel that more than a single release may be needed to allow establishment, so it is difficult to know at this point what a failure to recover specimens means.

CONCLUSIONS

Genetic analyses have confirmed our hypothesis that there are cryptic species hidden within specimens which morphologically appeared to be identical *A. epos*. In addition, what was thought to be *A. daanei* in Washington appears to be a different species from the California *A. daanei*. Given these genetic results in hand, it will be interesting to see if morphometric and SEM examination can differentiate between these cryptic species. Project cooperators plan to meet at the Pierce's disease symposium in San Diego to discuss our next steps. Obviously, field release and sampling should continue with the Minnesota strain *A. epos* to see what impact it may have on GWSS. If permits can be obtained allowing us to do so, we would also like to take GWSS egg outplants to Colorado to determine if *A.* n. sp nr *epos* two and three will parasitize GWSS eggs (these would be shipped back to the UCR's quarantine facility to allow parasitoids to emerge for confirmatory genetic analysis).

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LABORATORY AND FIELD EVALUATIONS OF NEONICOTINOID INSECTICIDES AGAINST THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT

Imidacloprid is still the most widely used neonicotinoid for the protection of grapevines against glassy-winged sharpshooter (GWSS) feeding and Pierce's disease (PD) transmission. This insecticide has now entered the generic age and within the past year, several new formulations of imidacloprid have been launched onto the market. To assist with grower acceptance of these new formulations, we are currently evaluating the uptake of different products in table and wine grapes. Bayer CropScience introduced Admire Pro to replace their original Admire 2F formulation. In Coachella Valley, the imidacloprid uptake profiles for vines treated with either Admire Pro or Admire 2F were similar, with peak uptake occurring within four days. In a further study, the uptake profile for Admire 2F was also consistent with a second soluble concentrate formulation (Nuprid 2F, marketed by Nufarm Americas Inc.).

We evaluated the performance of the neonicotinoid thiamethoxam (applied as Platinum) at three rates of application in a Temecula Valley wine grape vineyard. The concentrations of thiamethoxam in xylem fluid extracts were highest at the top application rates, and would provide good protection to vines against a sharpshooter infestation.

INTRODUCTION

Effective vector management through the use of the neonicotinoid insecticide, imidacloprid, has played a pivotal role in suppressing glassy-winged sharpshooter (GWSS) populations in California vineyards and citrus orchards (Castle et al., 2005; Byrne and Toscano, 2006). This in turn has greatly decreased the incidence of new Pierce's disease (PD) outbreaks in vineyards. With the expiry of the imidacloprid patent, there are now more formulations of this active ingredient becoming available to growers. To assist with grower confidence in the new products, we are evaluating their performances by measuring the uptake into vines (table and wine grapes) by extracting xylem fluid and quantifying the insecticide concentrations therein.

There are several insecticides within the neonicotinoid class with good systemic activity and each has its own distinct chemical properties that influence the efficacy with which the insecticide will work in the field. Systemic insecticides are commonly applied to vines through drip irrigation systems. This type of application is designed to deliver the insecticide close to the roots of the vines where more effective uptake into the plant xylem system can occur. In this way, systemic insecticides can directly exploit the xylophagous feeding behavior of the sharpshooter. Distribution of the insecticides within the plant xylem system can also provide more effective coverage of sharpshooter feeding sites and better persistence compared with foliar applications of the same product. As the number of available neonicotinoids increases, it is important to continue research efforts in order to better understand their behavior in California vineyards and to optimize their use by growers. Our studies in Coachella and Napa, for example, have shown that imidacloprid does not work consistently under all conditions experienced in California vineyards (Toscano and Byrne, 2005; Weber et al., 2005). We have, therefore, established a research program to examine the behavior of the different neonicotinoid insecticides within California vineyards.

In this report, we provide data on (1) the uptake and persistence of imidacloprid applied as different formulations, and (2) the impact of different rates of Platinum application on the uptake of thiamethoxam into grapevines.

OBJECTIVES

1. Determine the impact of soil type and irrigation on the uptake and residual persistence of neonicotinoid insecticides.
2. Develop an ELISA for the detection and quantification of dinotefuran residues within plant tissues.
3. Determine the uptake and persistence of imidacloprid, thiamethoxam and dinotefuran in grapevines in order to maximize protection of vineyards.