HOST SPECIFICITY OF THE MYMARID *ANAGRUS EPOS* GIRAULT, A PARASITOID OF CICADELLIDAE EGGS

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ABSTRACT

Anagrus epos Girault is a potential candidate for a classical biological control program targeting the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say) (Hemiptera: Cicadellidae). It is a gregarious parasitoid when reared on GWSS eggs. In choice tests, females successfully parasitized all developmental stages of GWSS eggs. Because mass production of GWSS is expensive and labor intensive, a factitious host that is more economical to produce is desirable to mass produce *A. epos* for colonization and augmentation efforts. In host specificity tests, females discriminated and oviposited into eggs of *Homalodisca liturata* Ball, *Erythroneura variabilis* Beamer, *Circulifer tenellus* (Baker), *Graphocephala atropunctata* (Signoret), *Amblysellus grex* (Oman), *Phoracantha recurva* Newman, and *Phoracantha semipunctata* (F.). *A. epos* successfully completed development in the eggs of *H. coagulata*, *H. liturata*, *E. variabilis*, *C. tenellus*, *G. atropunctata*, and *A. grex*.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), is a cicadellid native to the southern United States that was first detected in California in 1990 (Sorensen and Gill, 1996). It probably entered California via egg masses within nursery stock, and rapidly became responsible for spreading the bacterium *Xylella fastidiosa* Wells among vineyards throughout the Temecula area of Southern California (Blua et al., 1999). *Xylella fastidiosa* is the causal agent of Pierce's Disease (PD) in grapes (Davis et al., 1978), which is one of the most economically important crops in California ($2 billion/year), with over 853,000 acres distributed throughout the state (USDA, 2005). Current management of PD spread by GWSS includes removal of diseased grapevines (Hashim and Hill, 2003) and the use of insecticides and biological control agents to reduce GWSS populations in citrus and vineyards (Wendel et al., 2002; Hix et al., 2003).

Anagrus epos Girault (Hymenoptera: Mymaridae) is a newly introduced egg parasitoid brought to California from Minnesota, where it attacks eggs of *Cuerna fenestella* Hamilton (Hemiptera: Cicadellidae) (Triapitsyn and Rakitov, 2005). While in quarantine, *A. epos* was found to successfully parasitize GWSS eggs. It is now a potential candidate for mass production and releases against GWSS in California. In quarantine, *A. epos* proved to be a gregarious parasitoid, with up to 14 adult wasps emerging from each GWSS egg within an egg mass. In choice tests, *A. epos* females successfully parasitized all developmental stages of GWSS eggs (R. Krugner, unpublished data). Additionally, *A. epos* is known to parasitize the eggs of at least five different cicadellid species found in New Mexico, New York, and Colorado (Triapitysin, 1998), which suggests that if established, it may potentially use native California species as alternative hosts in areas where GWSS has not yet established or during periods when GWSS egg masses are unavailable.

Production of GWSS egg masses for insectary production of parasitoids is expensive, laborious, and space-demanding. Given this, an easier and less expensive method was sought to produce *A. epos* using the eggs of a factitious host. We also sought to better define the host specificity range of *A. epos*. Here, we report the results of tests to determine levels of *A. epos* female discrimination and host suitability.

MATERIALS AND METHODS

Establishment and Maintenance of the Anagrus epos *Colony*

An *A. epos* colony was established from individuals that emerged in the Entomology Department Quarantine Facility at the University of California at Riverside (UCR) on 8 June 2004 from eggs of *Cuerna fenestella* Hamilton (a native, univoltine proconiine sharpshooter) on *Solidago* sp. (goldenrod, *Compositae*) and *Zigadenus* sp. (death camus, *Liliaceae*) collected by R. Rakitov from 31 May to 1 June 2004 in Glyndon, Minnesota. Mated females were exposed to fresh GWSS eggs laid on *Euonymus japonica* L. f. leaves on 9 June 2004. The F_1 generation emerged on 29-30 June. A total of 11 females and 2 males were transferred to the senior author in October 2004, and reared for over 14 generations as described below on GWSS eggs until use in experiments.

The *A. epos* colony was maintained weekly by adding 5 to 10 *E. japonica* leaves (ca. 8 cm^2) each infested with a GWSS egg mass laid by field collected adults. Leaves were inserted through foam sheets, which were floated on 3 cm of water within the bottom of acrylic cages (10 x 10 x 15 cm) with fine mesh on the sides. Honey was streaked onto the interior walls of the cage and about 50 newly emerged females and 10 males were introduced into

each cage. Cages were held under constant room conditions (20-25°C, 60-80% RH, and 16:8 (L:D) h). Parasitoid development from egg to adult was completed within 20-30 d, and the rearing process was repeated upon adult eclosion.

Species Used for Host Preference and Suitability Testing

Insect species selected for the assays were chosen based on 1) host taxonomy (i.e., they share similar physiological properties and defense mechanisms); 2) shared ecology (i.e., unrelated hosts may share the same host plant or feeding niche); and 3) relative low cost to mass produce in controlled environment throughout the year.

Beet leafhopper eggs were obtained from a colony initiated from field collections from weedy vegetation near Coalinga, CA. The colony was maintained in a greenhouse on plants of potted sugar beet, *Beta vulgaris* var. *saccharifera* L. Sugar beet plants infested with beet leafhopper eggs were obtained by exposing plants to adult leafhopper females in a cage for 5 d.

Adults of the blue-green sharpshooter, *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), were obtained from a laboratory colony at UCR maintained on plants of potted basil, *Ocimum basilicum* L. Basil plants were kept inside rearing cages (60 × 60 × 60 cm). Rearing cages were kept in a greenhouse maintained at 26.7 ± 0.6°C, 24.8 ± 13.4% RH, and 16:8 (L:D) h. Basil plants infested with blue-green sharpshooter eggs were obtained by caging 5 adult females and 2 males per plant for 6 d.

Adults of the torpedo bug, *Siphanta acuta* (Walker) (Hemiptera: Flatidae), smoke tree sharpshooter, *Homalodisca liturata* Ball (Hemiptera: Cicadellidae), variegated leafhopper, *Erythroneura variabilis* Beamer (Hemiptera: Cicadellidae), and *Amblysellus grex* (Oman) (Hemiptera: Cicadellidae) were collected from citrus near San Juan Capistrano, citrus in Riverside, grapevines near Fresno, and tall fescue in Riverside, respectively. The torpedo bug, variegated leafhopper, and *A. grex* were confined on potted representatives of the plants from which they were collected for a 1 to 5-day ovipositional period. The smoke tree sharpshooters were confined on *E. japonica* plants to obtain newly laid eggs. Eggs of the eucalyptus longhorned borer, *Phoracantha recurva* Newman (Coleoptera: Cerambycidae), and *Phoracantha semipunctata* (F.) (laid on paper sheets) were obtained from a laboratory colony maintained at Agricultural Operations, UCR, as described by Hanks et al. (1993). Fresh eggs of the tobacco budworm, *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), oriental fruit moth, *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae), and *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) laid on glass slides, wax paper, and paper sheets, respectively, were obtained from laboratory colonies at UCR. Plants, leaves, and rearing substrates were taken into quarantine and tested under constant room conditions (20-25°C, 60-80% RH, and 16:8 (L:D) h).

Evaluation of Anagrus epos *Female Discrimination*

Eggs of all phytophagous species listed above were exposed to 48-hour old *A. epos* females under no-choice conditions. Substrates (e.g., leaves, paper, glass slides) infested with or supporting eggs were placed in petri dishes and exposed to a group of 10 *A. epos* females for a 1-hour observation period and were viewed through a video camera mounted on a stereoscope. After observation, wasps and leaves or ovipositional substrates were discarded. As a control, GWSS egg masses were exposed in a separate petri dish to *A. epos* females from the same test brood to ensure that females were mated, fertile, and fecund. All observations were conducted between 0900 and 1400 hours. After observation, host suitability tests were conducted as described below, with all 11 species independent of whether or not an ovipositional event was observed.

Host suitability tests

To determine if *A. epos* larvae could complete development on eggs of various host species, the following methods and host plant assemblages and cage setups were used for the host species indicated. Blue-green sharpshooter: five potted basil plants (10 cm high) infested with 1 to 6-day old blue-green sharpshooter eggs were covered with acrylic cages with fine mesh on the sides; Beet leafhopper: five potted sugar beet plants (five to six leaves) infested with 1 to 5-day old beet leafhopper eggs were covered with the acrylic cages; *A. grex*: four tall fescue bunches, *Festuca arundinacea* Schreb (30 to 50 leaves, 10 cm high), infested with 1 to 3-day old *A. grex* eggs were covered with acrylic cages; Variegated leafhopper: five potted Thompson seedless grapevines, *Vitis vinifera* L. (8 to 10 leaves), infested with 1 to 3-day old variegated leafhopper eggs were covered with a fine mesh screen; *S. acuta*: six citrus, *Citrus volkameriana* (Pasq.), leaves infested with a 2-day old *S. acuta* eggs were exposed as described above for *A. epos* colony maintenance; and smoke tree sharpshooter: 12 *E. japonica* leaves infested with smoke tree sharpshooter eggs were exposed as described for *S. acuta*. All other species were tested in their respective rearing substrate placed within the acrylic cages.

For each plant assemblage described above, 15 to 20 *A. epos* females were introduced to each cage and were allowed to oviposit for 72 h. After the exposure period, wasps were removed from the cages and discarded. Plants, leaves with eggs, and cages were thoroughly washed with water to remove remaining wasps. Cages were

observed daily until the first immature host and/or *A. epos* emerged, and every two days thereafter. We recorded the number and sex ratio of emerged *A. epos* and the total number of immature hosts that emerged within each cage.

RESULTS AND DISCUSSION

Evaluation of Anagrus epos *Female Discrimination.*

Of the 11 species studied, *A. epos* showed interest in and inserted its ovipositor into the eggs of seven species. *A. epos* females substantially reduced their walking speed and increased their frequency of turning and drumming the substrate with their antenna after approaching the area on the leaf where the eggs of GWSS, beet leafhopper, smoke tree sharpshooter, variegated leafhopper, and *A. grex* were located. Two of the 10 females exposed to beet leafhopper eggs located the eggs within the leaf in less than 40 s after exposure. After 10 to 12 s of drumming the eggs with the antenna, these females initiated an ovipositor behavioral response (i.e., drilling the leaf epidermis and egg shell followed by insertion of the ovipositor, abdominal contractions, and withdraw of the ovipositor) that lasted from 20 s to 3 min. Similar behaviors were observed for GWSS, smoke tree sharpshooter, variegated leafhopper, and *A. grex*, except that the entire host location and recognition process took approximately 10, 10, 56, and 20 min, respectively. Interestingly, *A. epos* females promptly perceived *P. recurva* and *P. semipunctata* eggs as potential hosts and performed the ovipositor behavioral response, which lasted from 1 to 3 min. Females performed the ovipositor behavioral response 2-3 times in each individual beetle egg, and sometimes returned to the same egg after visiting other nearby eggs. The cerambycid beetle eggs were relatively large (4 mm) compared to *A. epos* female adults (0.5 mm). This suggests that *A. epos* might have laid several eggs in each host egg. No ovipositor behavioral response was observed for blue-green sharpshooter, *S. acuta*, *H. virescens*, *G. molesta*, or *E. kuehniella* eggs, which suggests that these species were not perceived as potential hosts. We were unable to determine if the basil cuttings hosted any blue-green sharpshooter eggs during the observation. Females exposed to lepidopteran eggs spent most of their time resting or walking around the petri dish and never stopped to inspect any egg, whereas females exposed to GWSS eggs in a separate petri dish (the control) were observed to initiate oviposition soon after exposure. Overall, females in the act of oviposition permitted a second female to simultaneously oviposit in the same egg, and only briefly lifted their wings when the second female approached.

Host Suitability Tests

Anagrus epos successfully completed development in GWSS, blue-green sharpshooter, smoke tree sharpshooter, variegated leafhopper, beet leafhopper, and *A. grex* eggs. None of the exposed eggs of *S. acuta*, *H. virescens*, *G. molesta*, and *E. kuehniella* produced *A. epos* offspring, thereby confirming our host discrimination observations. Although *A. epos* females appeared to parasitize eggs of *P. recurva* and *P. semipunctata*, none of the attacked eggs produced *A. epos* offspring.

Developmental time in beet leafhopper eggs ranged from 18 to 53 d, although most individuals emerged within 35 days after oviposition. Among the five tested plants, we reared a mean of 38.0 ± 1.97 and 219.0 ± 16.43 males and females, respectively. The mean proportion of males and females (1.0 : 5.8) that emerged from each cage was relatively constant throughout the emergence period and, most importantly, at least one male emerged each day, which, under natural conditions, could reduce the likelihood that females would leave the natal site unmated. The estimated mean number of unparasitized eggs (as estimated by the number of leafhoppers that emerged) was 106.2 ± 21.92, which yields a mean parasitism rate of 71.4 ± 5.61%. There was a 2-day overlap between the last emerged leafhopper nymph and the first *A. epos* adult to emerge, which suggests that plants containing parasitized eggs can be safely placed in the field for mass releases without introducing leafhoppers into the system.

Developmental time from egg to adult of *A. epos* in variegated leafhopper eggs was approximately 23 d. Among the five tested plants, we reared a mean of 1.6 ± 0.81 and 4.2 ± 3.70 males and females per plant, respectively. A mean of 343.8 ± 63.99 leafhopper nymphs emerged per plant, which suggested a relatively low parasitism rate. However, microscopic examination of leaf material revealed the presence of numerous *A. epos* pupae that were unable to emerge from the eggs deposited along the leaf veins. Part of the grape leaves were dry during the *A. epos* emergence period because of intense feeding by variegated leafhopper nymphs and laboratory conditions, which suggests that *A. epos* requires fresh leaf tissue to be able to chew through the hard and dry leaf epidermis and emerge.

A. epos successfully developed in *A. grex* eggs, a cicadellid species collected in tall fescue, *F. arundinacea*, a Graminea commonly found in landscapes and parks throughout southern California. A mean of 6.2 ± 2.81 and 15.8 ± 9.12 males and females *A. epos*, respectively, and 44.6 ± 17.05 leafhopper nymphs emerged per plant, which resulted in a mean parasitism rate of 21.7 ± 7.28%. Developmental time from egg to adult was 25.7 ± 0.28 d and ranged from 19 to 35 d.

Future studies should investigate the relative importance of ecological habitat and host location processes influencing *A. epos* host utilization, which may assist in predicting the ecological risks associated with its introduction.

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REFERENCES

Blua, M.J., Phillips, P.A., Redak R. A. 1999. A new sharpshooter threatens both crops and ornamentals. California Agriculture 53, 22-25.

Davis, M.J., Purcell, A.H, Thompson, S.V. 1978. Pierce's disease of grapevines: isolation of the causal bacterium. Science 199, 75-77.

Hanks, L.M, McElfresh, J.S., Millar, J.G., Paine, T.D., 1993. *Phoracantha semipunctata* (Coleoptera: Cerambycidae), a serious pest of Eucalyptus in California: biology and laboratory-rearing procedures. Ann. Entomol. Soc. Am. 86, 96-102.

Hashim, J., Hill, B.L. 2003. Monitoring and control measures for Pierce's disease in Kern County, and epidemiological assessments of Pierce's disease, pp. 95-98. *In* M. A. Tariq, S. Oswalt, P. Blincoe, R. Spencer, L. Houser, A. Ba, and T. Esser [eds.]. Proceedings of CDFA Pierce's Disease Research Symposium, 8-11 December 2003, Coronado, CA, Copeland Printing, Sacramento, CA.

Hix, R., Toscano, N., Gispert, C. 2003. Area-wide management of the glassy-winged sharpshooter in the Temecula and Coachella Valleys, pp. 292-294. *In* M. A. Tariq, S. Oswalt, P. Blincoe, R. Spencer, L. Houser, A. Ba, and T. Esser [eds.]. Proceedings of CDFA Pierce's Disease Research Symposium, 8-11 December 2003, Coronado, CA, Copeland Printing, Sacramento, CA.

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. Transactions of the American Entomological Society 124 (2), 77-112.

Triapitsyn, S.V., Rakitov, R.A. 2005. Egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of *Cuerna* sharpshooters (Hemiptera: Cicadellidae) in the USA, Posters P11-P12. *In*: Poster Abstracts P1-P43, 12th International Auchenorrhyncha Congress and 6th International Workshop on Leafhoppers and Planthoppers of Economic Significance, University of California, Berkeley, 8-12 August 2005. Available online (.pdf) at http://nature.berkeley.edu/hoppercongress/.

Sorensen, J.T., Gill, R.J. 1996. A range extension of *Homalodisca coagulata* (Say) (Hemiptera: Clypeorrhyncha: Cicadellidae) to southern California. Pan-Pacific Entomologist 72, 160-161.

United States Department of Agriculture. 2004. California Grape Acreage Report. ftp://www.nass.usda.gov/pub/nass/ca/rpts/acreage/200503grpac.pdf.

Wendel, L., Ciomperlik, M, Bartels, D., Luvisi, D., Elms, D. 2002. The area-wide pest management of glassy-winged sharpshooter in Kern County, pp. 302-307. *In* M. A. Tariq, S. Oswalt, P. Blincoe, and T. Esser [eds.], Proceedings of CDFA Pierce's Disease research symposium, 15-18 December 2002, Coronado, CA. Copeland Printing, Sacramento, CA.

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