OVIPOSITION AND NATIVE PARASITOIDS OF THE BLUE-GREEN SHARPSHOOTER, AND HOST SPECIFICITY OF *GONATOCERUS ASHMEADI* ON THE SMOKETREE SHARPSHOOTER AND THE BLUE-GREEN SHARPSHOOTER

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ABSTRACT

The studies outlined below represent two years of a three year project. We have determined the oviposition preferences of *Graphocephala atropunctata* (Signoret) (Hemiptera: Cicadellidae), blue-green sharpshooter, (BGSS) on wild grape, have documented its associated egg parasitoids, and provide data on host specificity of *Gonatocerus ashmeadi,* a parasitoid being used as part of the classical biological control program against glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* Say (Hemiptera: Cicadellidae) on the target's congener, the native *Homalodisca liturata* Ball, smoketree sharpshooter (STSS), as well as the BGSS. To determine the oviposition of female BGSS, a survey was conducted on southern California wild grape, *Vitis girdiana* Munson (Vitaceae) growing near Temecula, CA in August 2003 and 2004 where populations of BGSS were known to occur. Female BGSS oviposited into new growth, primarily the succulent tendrils and stems. Two parasitoids, *Gonatocerus latipennis* Girault and a *Polynema* sp. (Hymenoptera: Mymaridae) were reared from BGSS eggs. Deployment of sentinel plants and reciprocal tests were implemented to further confirm the parasitization of BGSS eggs by these parasitoids. Collectively the *Polynema* sp. and *Gonatocerus latipennis* constitute the first documented natural enemies of BGSS eggs. Additional studies, commencing in January 2004, of the activity of BGSS and its parasitoids in southern California is currently underway. Blue-green sharpshooter adult activity reached its peak in July while bi-weekly samples of wild grape canes and tendrils revealed peak emergence of BGSS nymphs and parasitoids occurred from mid-July to mid-August. These peaks were found to be significantly correlated. Choice and no-choice tests of *Gonatocerus ashmeadi* Girault and *G. fasciatus*, (Hymenoptera: Mymaridae) parasitoids of GWSS with the native smoketree sharpshooter (STSS), and BGSS eggs as part of a retrospective non-target impact assessment have yielded interesting results. Parasitism of STSS eggs by *G. ashmeadi* and *G. fasciatus* does not appear to be significantly different when compared to the GWSS control in no-choice experiments. Additionally, it appears *G. ashmeadi* exercises no preference of host eggs for parasitization when presented with a choice of STSS and GWSS simultaneously.

INTRODUCTION

Examining possible non-target effects of biological control agents is becoming a more common requirement for many biological control programs targeting arthropod pests. Currently, for classical biological control of weeds, the Wapshere centrifugal method provides an excellent means for eliminating possible natural enemies that could cause harm to non-target plants. However, a rigorous, reliable, and broadly applicable testing standard for arthropod biological control is currently lacking. No-choice and choice testing strategies are a common way to test for possible non-target effects of new biological control organisms. However, these lab studies are often carried out in small testing arenas where the study organism is forced onto the host which may be adequate for determining physiological host range but may seriously overestimate its ecological host range in nature. Our research involves the use of rigorous testing strategies utilizing standard Petri dish test arenas, coupled with larger-scale entire plant test arenas in no-choice and choice comparisons. As retrospective studies in ongoing biological control programs can yield valuable information on non-target impacts, we chose the GWSS classical biological control program in California as a model for our non-target studies. We are examining the possible non-target impacts of the self-introduced *G. ashmeadi* and the recently introduced *G. fasciatus* Girault, egg-parasitoids of GWSS, and three sharpshooters native to California, U.S.A.: (1) STSS (2) BGSS; and (3) green sharpshooter (GSS), *Draeculocephala minerva* Ball (Hemiptera: Cicadellidae). Our experiments with small-scale Petri dish studies and larger-scale full plant studies are supplemented with sentinel plants and habitat surveys to determine the invasiveness of GWSS parasitoids.

OBJECTIVES

1. Classify the native egg-parasitoid fauna in California associated with sharpshooters native to California, primarily the smoketree sharpshooter (STSS): *Homalodisca liturata* Ball (Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Proconiini), blue-green sharpshooter (BGSS): *Graphocephala atropunctata* (Signoret), and green sharpshooter (GSS): *Draeculocephala minerva* Ball (the latter three, all Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Cicadellini).
2. Assess the possible non-target impacts of *Gonatocerus ashmeadi* and *G. fasciatus*, parasitoids being used for the classical biological control of GWSS, on the above mentioned native sharpshooters.

RESULTS

Oviposition Survey

Please see Hoddle (2004) for a detailed overview of BGSS parasitoids and oviposition on *V. girdiana*. Ten entire grape canes were sampled on 14 August 2003. These canes were cut into thirds (apical, middle and basal), then placed into 10 cm of water in a Mason jar which left approximately 25 cm of cane exposed for emergence of nymphs and parasitoids. Canes and mason jars were then placed into three separate cages, according to their stem position. Cane sections were examined daily for emergence. In total, two BGSS nymphs and 16 *Polynema* sp. emerged from the canes. As there were so few insects emerged from these cane sections, the stems, leaves, petioles and tendrils were examined under the microscope for recent emergence holes by BGSS nymph and associated parasitoids. A total of 65 emergence holes were counted. The majority of emergence holes were on the apical stems (n = 37) and on tendrils (n = 6, 13, 7, for apical, middle and basal portions, respectively) occurring along the length of the entire canes. Only two emergence holes were counted from leaf petioles and none were counted from middle and basal stems and leaves. This survey was repeated in 2004. A total of two BGSS nymphs and 52 *Polynema* sp. emerged from the 16 full canes sampled on 6 August 2004. A total of 95 emergence holes were counted. The majority of emergence holes were on the apical stems and on tendrils occurring along the length of the canes. Fifteen emergence holes were counted from leaf petioles (not shown) and only one was counted from a leaf midrib from a middle 45cm cane section. The cumulative results of this survey are presented in Figure 1. Cane sections of the same substrate type with the same letter are not significantly different (p>0.05). Substrate type within the same cane section with the same letter are not significantly different (p>0.05). Statistical analysis was carried out by use of Kruskal-Wallis one-way ANOVA (Proc nonpar1way, SAS Institute 1999) for on average ranks for cane sections and substrate types.

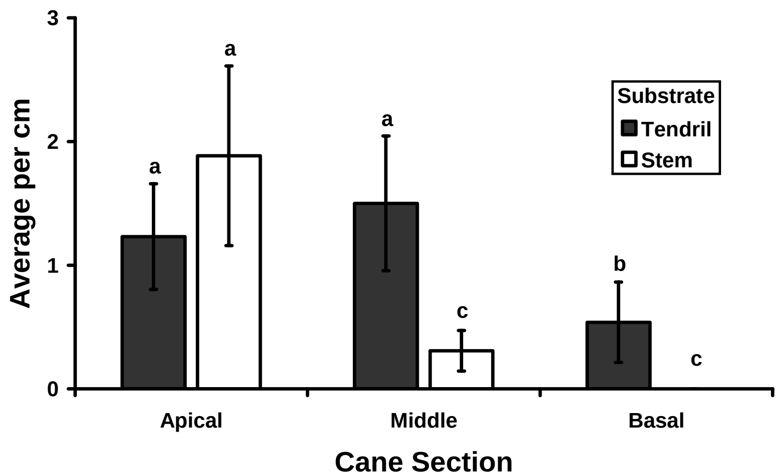

Figure 1. BGSS oviposition inferred from average nymph and *Polynema* sp. emergence per centimeter of grape cane.

Sentinel Plant Study

To confirm the host association of the emerged parasitoids with the BGSS, sentinel plants were exposed to BGSS lab colonies for 3 days to allow for oviposition. Plants were removed from the colonies and transported to the oviposition survey site to allow for parasitization of BGSS eggs by native parasitoids. After three days, the deployed plants were brought back from the field, cleaned of any insects and placed into separate cages. Plants were observed daily for any emerging insects. A combined total of 197 BGSS and *Polynema* sp. emerged from the five sentinel plants in 2003. Of these, 55 were BGSS nymphs and 142 were *Polynema* sp. (54 males, 88 females). Parasitism rates of BGSS eggs by *Polynema* sp. ranged from 33% on Chrysanthemum to 78% and 86% on wild grape and basil, respectively. A total of 23 BGSS and *Polynema* sp. emerged from the six sentinel plants in 2004. Of these, five were BGSS nymphs and 18 were *Polynema* sp. (2 males, 16 females). Parasitism rates of BGSS eggs by *Polynema* sp. ranged from 50% to 100%.

Reciprocal Tests

To confirm association of the correct parasitoid species with BGSS, sentinel plants bearing BGSS eggs were deployed at field sites and returned to the lab where parasitoids were reared out. Parasitoids that emerged from 2004 sentinel plant studies were captured into small vials and released into cages containing a basil plant with <48 hr old BGSS eggs and allowed 48 hrs to parasitize the eggs. Parasitism was confirmed by emergence of 11 male *Polynema* sp. No *G. latipennis* emerged from the sentinel plants, and thus no reciprocal tests were conducted on the BGSS with this parasitoid. Separate emerged *Polynema* sp. exposed to GWSS and STSS eggs on citrus and Chrysanthemum leaves in Petri dishes yielded no parasitism by this native parasitoid.

BGSS and Parasitoid Activity

A total of 12 yellow sticky card traps (11 x 15 cm), were placed at the 2003 oviposition survey site to monitor BGSS adult and parasitoid flight activity. Traps were set up on 9 January 2004 and collected at bi-weekly intervals. Peak trap catch of BGSS adults occurred over the two week period of 11 June to 25 June 2004. Additionally, as soon as wild grape had sprouted and was available for collection, starting on 16 April 2004, twelve 30 cm cane sections were collected at bi-weekly sampling intervals. Tendrils were cut from the cane and placed into individual Petri dishes while stems were placed into dual 50 dram vials (25 cm of cane above water to allow for emergence). Plant material was checked daily for emergences of nymphs and parasitoids. Peak emergence of BGSS nymphs and parasitoids was spread over a four week period from 24 July to 20 August 2004. Emergence data from 14 May 2004 to 29 October 2004 was highly correlated to trap catch data from 16 April 2004 to 1 October 2004 (Pearson Correlation Coeff. = 0.92, p < 0.0001, Proc corr, SAS Institute, 1999). For those time periods trap data explains 84% of variation in emergence data and vice versa. Data compilation for 2005 is still in progress however some of the results are shown below in Figure 2.

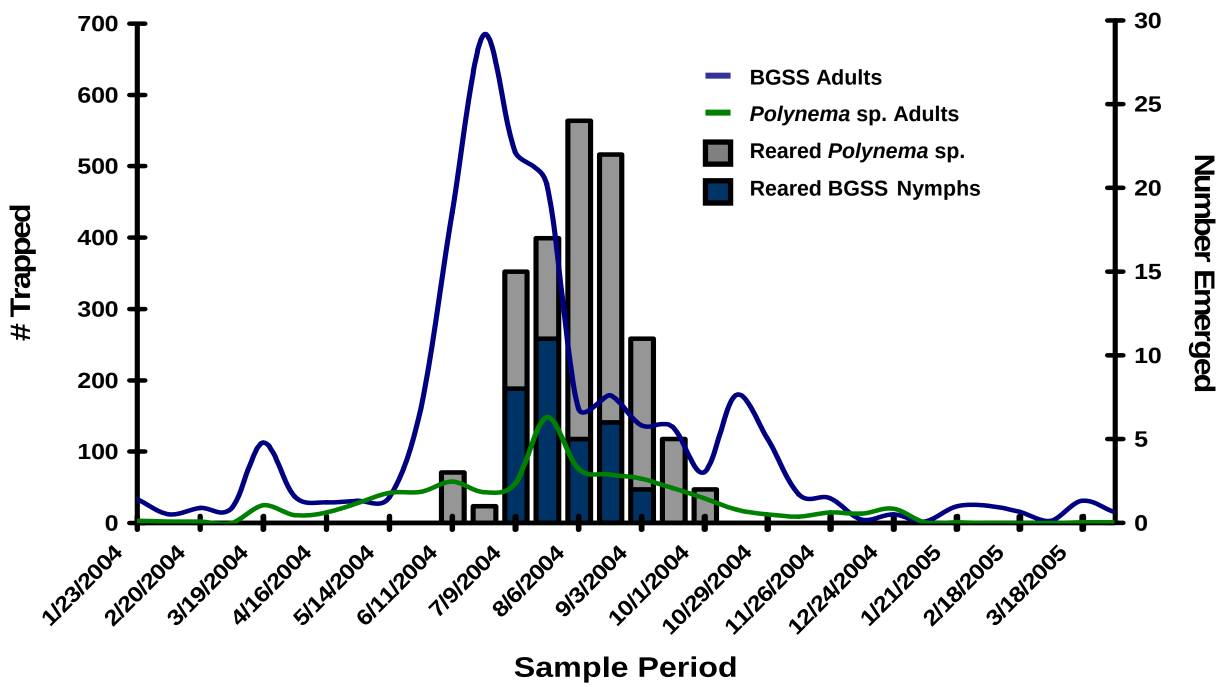

Figure 2. Flight activity and phenology of BGSS and *Polynema* sp.

Host specificity testing

Choice and no-choice tests were conducted with *G. ashmeadi* and *G. fasciatus* on BGSS and STSS eggs using GWSS eggs as a control. Tests were conducted on two scales, micro (= Petri dish, 100 x 15 mm) and macro (= full plant, approximately 30 cm height), using single, one day old, mated, honey water-fed *G. ashmeadi* or *G. fasciatus*. BGSS eggs were not tested at the micro scale nor were they tested in a choice arena. Each test was conducted utilizing two different host plants. For STSS, Eureka Lemon and Jojoba were used, while sweet basil and wild grape (*V. girdiana*) were utilized for the BGSS. In Petri dish choice tests, the parasitoid was exposed to approximately 20 of each STSS and GWSS eggs simultaneously. Full plant choice tests included approximately 40 of each STSS and GWSS eggs presented simultaneously to the parasitoid on one type of host plant at a time. For no-choice testing each parasitoid was supplied approximately 40 target eggs. All target eggs were less than 48 hours of age. In all tests the parasitoid was provided honey water as a food source and allowed 24 hr to parasitize the eggs before removal from the testing arena. Percent parasitism of egg masses ranged from 0-100% for both STSS and GWSS. Many replicates are still in progress; however, preliminary results of completed tests are shown below for *G. ashmeadi* (Figure 3). Parasitism of STSS eggs by *G. ashmeadi* and *G. fasciatus* does not appear to be statistically different as compared to the GWSS control in no-choice experiments. Additionally, it appears *G. ashmeadi* exercises no preference of host eggs for parasitization when presented with a choice of STSS and GWSS simultaneously. No parasitism of BGSS eggs by *G. ashmeadi* or *G. fasciatus* was observed for either the sweet basil or wild grape host plants.

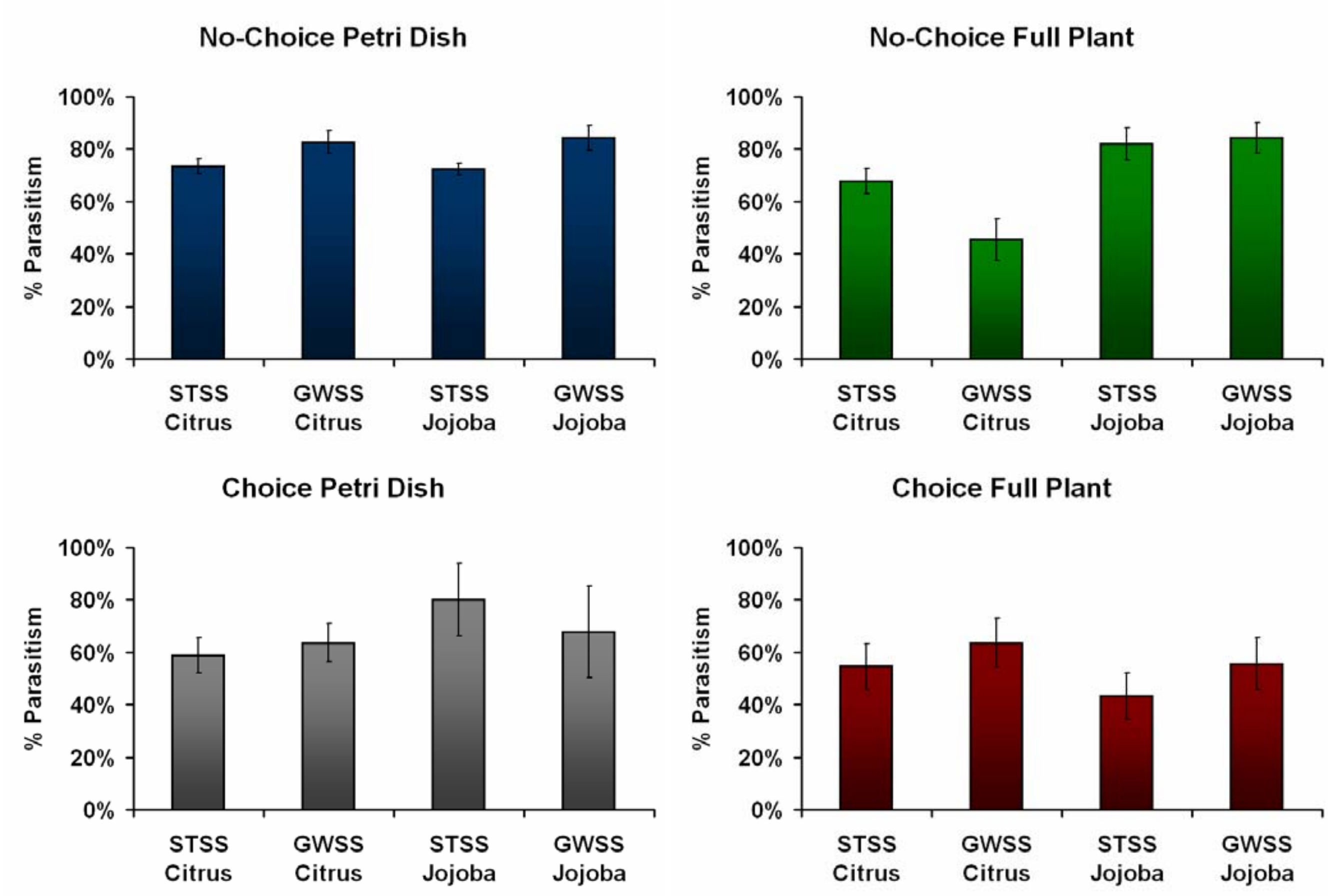

Figure 3. Percent parasitism of STSS and GWSS eggs by *G. ashmeadi* in choice and no-choice studies.

CONCLUSIONS

While results for laboratory choice and no-choice tests with *G. ashmeadi* and *G. fasciatus* are still being tabulated for STSS and BGSS, preliminary data shows neither parasitoid will parasitize BGSS eggs, but will parasitize STSS eggs. In fact, STSS egg masses appear to be attacked as readily as the GWSS control in no-choice tests at both Petri and whole plant scales and with no preference for either host eggs in choice tests at both scales. Given the substantial availability of GWSS eggs, these parasitoids may impact the native *Ufens* spp. (Hymenoptera: Trichogrammatidae) parasitoid complex if large numbers of *G. ashmeadi* spill out of GWSS infested areas and attack STSS eggs, the preferred host for *Ufens* spp. Furthermore, *G. ashmeadi* emerging from STSS eggs are smaller and less fecund than those developing from GWSS eggs (N. Irvin unpublished data – but see this report). We would speculate these 'runts' may have an overall reduced fitness, and that STSS eggs may ultimately be a dead-end host for *G. ashmeadi*, especially if no selection of evolutionary significance occurs for use of STSS eggs. However, if these parasitoids were to establish in large numbers in the xeric habitats where STSS is most abundant, and if these larval parasitoids are able to out-compete larval *Ufens* spp., then we might expect a drastic impact on the natural enemy fauna of STSS in desert regions. For example, the establishment of these exotic parasitoids in the fragile ecosystems of the desert oases at Joshua Tree National Park, where the STSS and *Ufens* spp. coexist in a delicate balance, could have significant impacts. Presently, we suspect that *G. ashmeadi* and most likely *G. fasciatus* are unlikely to physiologically withstand the harsh environments of desert areas of eastern California, but the possibility and the consequences of such an incursion, should it occur, are worth consideration.

Our research approach with GWSS parasitoids attempts to include not only the physiological, ecological, but also the temporal and spatial elements in determining possible native sharpshooter (and associated native parasitoids) non-target effects. Via choice and no-choice testing at two scales, parasitoid behavioral studies in the field, non-target habitat monitoring and natural enemy classification, and by determining oviposition, egg, and habitat characteristics of the possible non-target species, we are obtaining important information for retroactively assessing the possible risk posed by these exotic natural enemies of GWSS to native members of the receiving ecosystem.

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