NON-TARGET IMPACT OF GWSS PARASITOIDS, *GONATOCERUS ASHMEADI* AND *G. FASCIATUS*, ON SHARPSHOOTERS NATIVE TO CALIFORNIA, WITH NOTES ON INDIGENOUS PARASITOIDS OF BGSS AND GSS

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ABSTRACT: A rigorous testing strategy involving choice and no-choice test arenas was developed to explore the potential non-target impacts of classical biological control agents. The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), biological control program was studied as a model system for analyzing choice and no-choice host preferences for natural enemies and also for performing retrospective non-target impact assessments. *Gonatocerus ashmeadi* Girault and *G. fasciatus* Girault (both Hymenoptera: Mymaridae), egg parasitoids of the exotic GWSS, were tested against three potential non-target sharpshooters indigenous to southern California. Work conducted here showed that the solitary *G. ashmeadi* was able to successfully parasitize eggs (i.e., viable progeny resulted) of smoke-tree sharpshooter (STSS), *Homalodisca liturata* Ball, but not eggs of the blue-green sharpshooter (BGSS), *Graphocephala atropunctata* (Signoret), or the green sharpshooter (GSS), *Draeculacephala minerva* Ball (all Hemiptera: Cicadellidae). *Gonatocerus ashmeadi* exercises no parasitization preference when presented with a choice of STSS and GWSS eggs simultaneously. The gregarious *G. fasciatus* parasitized eggs of STSS and GSS, but not eggs of the BGSS. BGSS and GSS eggs were collected from the field and reared to catalog their indigenous egg parasitoid fauna. Any parasitoids reared from these eggs were reciprocally exposed to 'clean' lab colony eggs. Two parasitoids, *Gonatocerus latipennis* Girault and a *Polynema* sp. (Hymenoptera: Mymaridae) were confirmed as parasitoids of BGSS eggs. Three parasitoids, *Gonatocerus mexicanus* Perkins and two unidentified trichogrammatids were confirmed as parasitoids of GSS eggs.

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 centrifugal method provides a robust theoretical framework for identifying potential natural enemies that could cause harm to non-target plants. However, a rigorous, reliable, and universally applicable testing standard for arthropod biological control with a strong theoretical basis 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. Under these conditions, efficacious natural enemies may be unnecessarily eliminated from the candidate natural enemy pool as being insufficiently host-specific. To more accurately determine the host range of a natural enemy our research involved 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 impact studies. We are examining the possible non-target impacts of the self-introduced and omnipresent *G. ashmeadi* and the recently introduced *G. fasciatus*, both egg parasitoids of GWSS, on three sharpshooters native to California, U.S.A.: the STSS (native congener to GWSS), BGSS, and GSS. Our experiments with small-scale Petri dish studies and larger-scale full plant studies were supplemented with deployment of sentinel plants bearing eggs from laboratory colonies of BGSS or GSS and habitat surveys to determine the invasiveness of GWSS parasitoids into habitats occupied by BGSS or GSS.

OBJECTIVES: 1.) To classify the native egg-parasitoid fauna in California associated with sharpshooters native to California, primarily the smoke-tree 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 two, all Hemiptera: Clypeorrhyncha: Cicadellidae: Cicadellinae: Cicadellini). 2.) To assess the possible non-target impacts of *Gonatocerus ashmeadi* Girault and *G. fasciatus* (both Hymenoptera: Mymaridae), parasitoids being used for the classical biological control of GWSS, on the above mentioned native sharpshooters.

RESULTS:

Indigenous parasitoids of BGSS and GSS: Eggs of BGSS and GSS were collected from natural habitats in California and held at laboratory temperatures to rear egg-parasitoids. Sentinel eggs from lab colonies of either BGSS or GSS were placed in the field to expose eggs to resident parasitoids. Parasitoids reared from field collected or sentinel eggs were exposed to 'clean' eggs from lab colonies to confirm their host association with the proposed native sharpshooter. Two egg-parasitoids, *Gonatocerus latipennis* and a *Polynema* sp., were reared from field collected BGSS eggs. *Polynema* sp. was confirmed as a parasitoid of BGSS. Three parasitoids, *Gonatocerus mexicanus* and two unidentified trichogrammatids were reared from field collected GSS eggs. All three were confirmed as parasitoids of GSS eggs via reciprocal attacks on "clean" eggs from the GSS lab colony.

Host specificity testing: Choice and no-choice tests were conducted with *G. ashmeadi* and *G. fasciatus* on BGSS, GSS, 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*. Micro scale tests were used to estimate the physiological host range of the parasitoid by reducing the area of search and increasing host contact thereby forcing the parasitoid onto a host. By contrast, the macro scale tests were utilized to estimate the ecological host range of the parasitoid by incorporating host finding on an entire plant thereby enabling the parasitoid to use a greater part of its searching repertoire for assessing host suitability. BGSS eggs have not yet been tested at the micro scale. Each test was conducted utilizing two different host plants for each of the sharpshooters examined. An effort was made to include at least one native or naturalized California host plant. Eureka Lemon (*Citrus limon* (L.) Burm.f. cv. 'Eureka'; Sapindales: Rutaceae) and jojoba (*Simmondsia chinensis* (Link) Schneid.; Euphorbiales: Simmondsiaceae) were used for STSS, sweet basil (*Ocimum basilicum* L.; Lamiaceae: Lamiales) and wild grape (*Vitis girdiana* Munson; Rhamnales: Vitaceae) for BGSS, and milo (*Sorghum bicolor* L. Moench; Cyperales: Poaceae) and rescuegrass (*Bromus catharticus* Vahl.; Cyperales: Poaceae) for GSS. In choice tests, the parasitoid was exposed to approximately 20 eggs each of the test species and control on the same host plant type simultaneously. For no-choice testing, each parasitoid was supplied approximately 40 target eggs. All target eggs were < 48 h of age. In all tests, the parasitoid was provided honey water as a food source and allowed 24 h to parasitize eggs before removal from the testing arena.

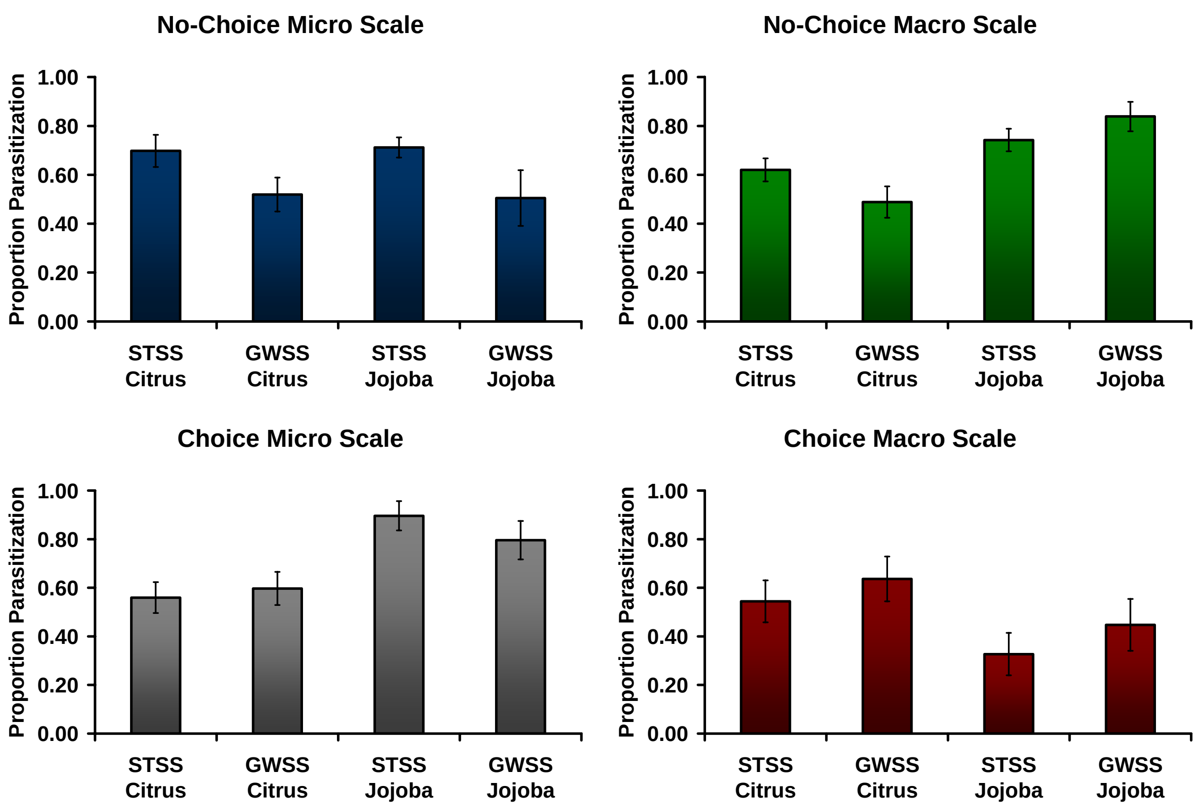

Fig. 1. Proportion parasitism of STSS and GWSS eggs by *G. ashmeadi* in choice and no-choice studies.

For no-choice micro scale tests, proportion parasitization of STSS eggs by *G. ashmeadi* appears to be higher than that of GWSS eggs for both host plants tested (Fig. 1). This trend appears to hold true for no-choice macro scale tests on lemon, but not for jojoba. For both micro and macro choice arenas, there does not appear to be any difference in proportion parasitism when *G. ashmeadi* is offered a choice between GWSS and STSS on either host plant. *G. ashmeadi* did not parasitize BGSS eggs on basil or grape in either macro choice or no-choice test arenas, and this parasitoid did not parasitize GSS eggs on milo or rescuegrass in any of the four test arenas.

Preliminary data shows that *G. fasciatus* will parasitize STSS eggs in all four test arenas (Fig. 2) and can produce 2-5 offspring per egg. It did not parasitize BGSS eggs on basil or grape in either macro choice or no-choice tests. *G. fasciatus* parasitized GSS eggs (producing two offspring per egg) on rescuegrass in both choice and no-choice micro scale test arenas, but did not parasitize eggs in either macro choice or no-choice tests. Many replicates for *G. fasciatus* are in progress for both STSS and GSS, thus results are preliminary.

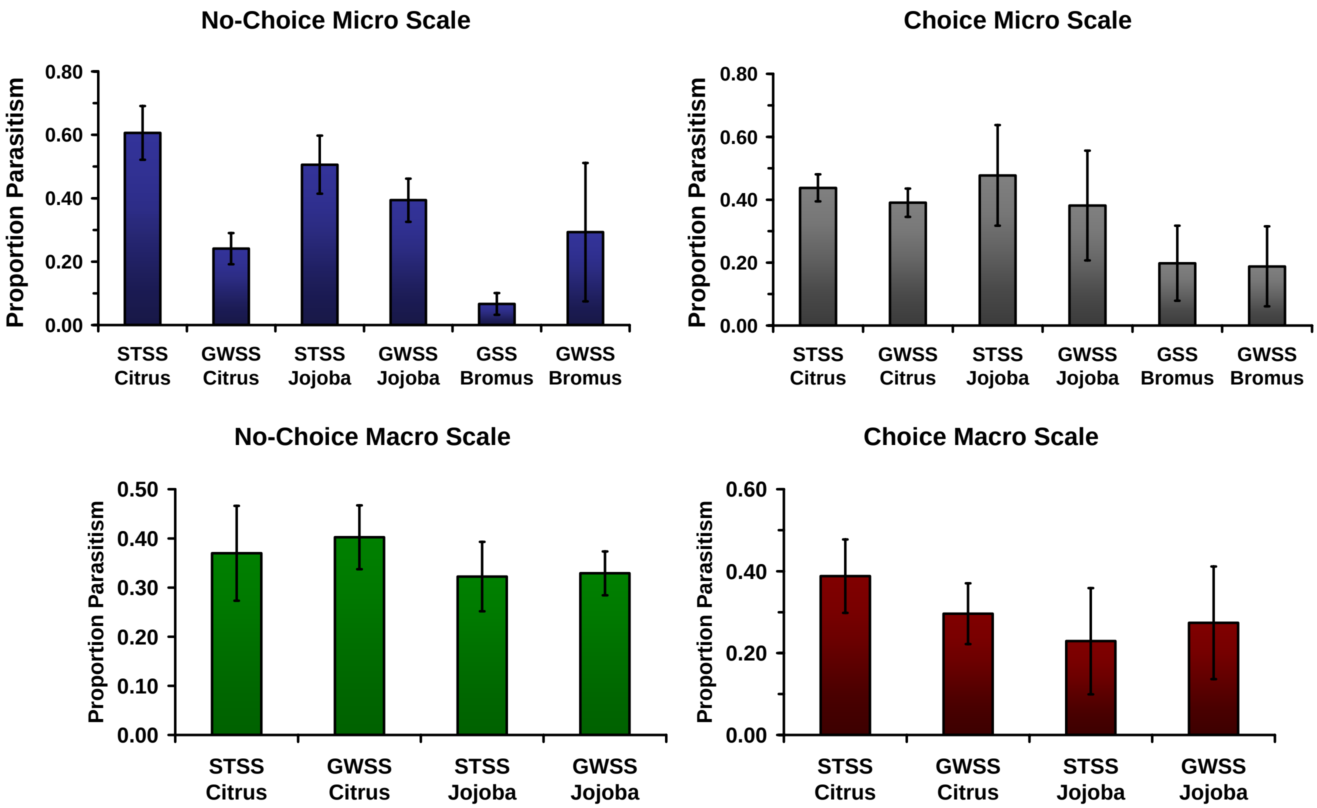

Fig. 2. Proportion parasitism of STSS, GSS, and GWSS eggs by *G. fasciatus* in choice and no-choice studies.

CONCLUSIONS: BGSS, GSS, and STSS are all vectors of Pierce's Disease and documentation of the indigenous natural enemy complex for these sharpshooters is an essential step when developing sustainable control options that rely on resident natural enemies for pest control. Now that progress has been made on identifying the indigenous parasitoid fauna associated with Xylella-vectoring native sharpshooters it may be possible to conserve or augment populations of these natural enemies in future management programs. Additionally, documentation of these parasitoids will allow for subsequent research on the depth of any indirect non-target impacts associated with the GWSS biological control program.

It is interesting that the solitary egg-parasitoid, *G. ashmeadi*, will parasitize more STSS eggs than it will GWSS eggs in a no-choice test arena. STSS eggs are smaller then GWSS eggs (Al-Wahaibi 2004) and *G. ashmeadi* offspring are smaller and less fecund from STSS eggs than those reared from GWSS eggs (N. A. Irvin unpublished data). It is possible the parasitoid is aware that the offspring will be smaller and less fit, so the mother may compensate by parasitizing more of the smaller host eggs. However, when offered a choice between the target and non-target eggs, there is no difference in parasitism regardless of host plant. This might suggest that the parasitoid is distributing her chances of offspring survival evenly between the two hosts. 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. 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 this parasitoid were to establish in large numbers in the xeric habitats where STSS is most abundant and where GWSS is relatively rare or absent, and if *G. ashmeadi* larvae are able to out-compete *Ufens* spp. larvae, then we might expect an impact on the native natural enemy fauna of STSS in desert regions. 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.

While results for the gregarious *G. fasciatus* are still in progress, it will be interesting to see if similar trends for STSS parasitism are observed for this parasitoid as were observed for *G. ashmeadi*. Since *G. fasciatus* has parasitized GSS eggs successfully producing two female offspring per GSS egg, despite the egg length being approximately one-half the length of a GWSS egg, it suggests GSS is a physiologically acceptable host for *G. fasciatus*. However, no *G. fasciatus* were reared from field collected egg masses, and the parasitoid has not parasitized GSS eggs at the macro scale in either choice or no-choice arenas. This suggests that although *G. fasciatus* is physiologically capable of parasitizing GSS eggs and will attack eggs from this species in a Petri dish, it is unlikely that GSS eggs are an ecologically suitable host for this parasitoid as this parasitoid has failed to parasitize GSS on entire plants. Consequently, it is unlikely this parasitoid will be a significant threat to indigenous egg-parasitoids of GSS.

Our research approach with GWSS parasitoids has attempted to include physiological, ecological, 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 field surveys, 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 two exotic natural enemies of GWSS (*G. ashmeadi* and *G. fasciatus*) to native members of the receiving ecosystem.

REFERENCES:

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