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# Host specificity testing of *Gonatocerus* spp. egg-parasitoids used in a classical biological control program against *Homalodisca vitripennis*: A retrospective analysis for non-target impacts in southern California

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**Abstract**

Classical arthropod biological control programs lack a universally adopted, broadly utilitarian, and standardized risk assessment testing strategy for precisely estimating physiological and ecological host range of potential biological control agents. We implemented a rigorous host specificity testing protocol for estimating potential physiological and ecological risk to non-target species posed by exotic parasitoids utilized in a classical biological control program and subsequently evaluated it in terms of efficacy at estimating realized risk. This testing strategy was evaluated at two environmental scales, micro-scale Petri dish studies and macro-scale cage studies, with choice and no-choice host options presented on multiple plant species to two related egg parasitoids with dissimilar life history strategies. The solitary *Gonatocerus ashmeadi* Girault and gregarious *G. fasciatus* Girault (Hymenoptera: Mymaridae) are non-native egg-parasitoids of the exotic *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), and were introduced as part of a classical biological control program in southern California, USA. The parasitoids' physiological and ecological host ranges were estimated on three non-target indigenous sharpshooters and results were compared with observed non-target impacts in the field. Laboratory tests with *G. ashmeadi* revealed *Homalodisca liturata* Ball (Hemiptera: Cicadellidae) eggs were a physiologically and ecologically acceptable host; *Graphocephala atropunctata* (Signoret) and *Draeculacephala minerva* Ball (Hemiptera: Cicadellidae) eggs were not acceptable hosts. Tests with *G. fasciatus* revealed both *H. liturata* and *D. minerva*, but not *G. atropunctata* eggs, were a physiologically acceptable host. Only *H. liturata* eggs were determined to be an ecologically acceptable host for *G. fasciatus*. Non-target parasitism of *H. liturata* eggs by *G. ashmeadi* in southern California habitats was determined. Field surveys reported here failed to reveal parasitism of *G. atropunctata* or *D. minerva* eggs by either *G. ashmeadi* or *G. fasciatus* in the respective native habitats in southern California.
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## 1. Introduction

Accurately testing for and verifying the existence and magnitude of non-target impacts of an arthropod biological control agent (ABCA) has become a significant concern in the past two decades for biological control programs targeting arthropod pests. This disquiet has arisen because the environmental safety of this technology for controlling invasive pests has been challenged thereby generating considerable debate and research interest in classical biological control with exotic natural enemies (Howarth, 1983, 1991; Sands, 1997; Van Driesche and Hoddle, 1997; Withers et al., 1999; Follett and Duan, 2000; Wajnberg et al., 2001; Van Driesche, 2002; van Lenteren et al., 2003; Van Driesche and Reardon, 2004; Babendreier et al., 2005; Bigler et al., 2006). As successful classical biological control programs can reduce the need for pesticides by specifically targeting a pest species and aid in restoring a habitat to similar

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conditions as those observed prior to the pest introduction (Hoddle, 2004a,c), biological control can be seen as providing environmentally sensitive and economically sound solutions to invasive pest problems (Pickett et al., 1996; Guitierrez et al., 1999). Although well documented and serious non-target effects are thought to be a relatively rare occurrence for biological control programs implemented in the last forty years (Henneman and Memmott, 2001; Hoddle and Syrett, 2002), it is well recognized that exotic ABCAs have high host and habitat fidelity to minimize collateral damage to non-target organisms (Bellows, 2001; Hoddle, 2004b). However, universal consensus on an appropriate testing strategy for ABCAs to eliminate potential unwanted non-target impacts has not been reached, and arguably, may not be possible because of highly varied and environmentally influenced behaviors exhibited by many arthropod natural enemies, especially parasitoids (Duan and Messing, 2000; Messing et al., 2006). In stark contrast, classical biological control of weeds has well-developed and almost universally applicable host specificity and risk analysis testing strategies. The centrifugal-phylogenetic method provides one widely used testing framework for eliminating possible natural enemies that could cause harm to non-target plants (Wapshere, 1974, 1975). Conversely, adoption of a similar phylogenetically-based strategy for arthropods is often not practical because, of unstable taxonomy of target or natural enemy groups, habitat influenced behaviors that are difficult to assess in the laboratory, and the overwhelming numbers of possible, often rare, poorly known, and difficult to mass rear native non-target arthropods has prevented the use of the same or similar phylogenetic-based strategy in arthropod biological control (ABC) (Hoddle, 2004a). In response to concern about non-target impacts associated with the introduction of exotic biological control agents (Louda et al., 2003; Louda and Stiling, 2004; Simberloff and Stiling, 1996), several methodologies have been proposed to more accurately determine risk in ABC (see van Lenteren et al., 2006; and references therein). Of those methods proposed, no-choice and choice experiments are approaches promoted for host range determination in ABC programs (Withers and Mansfield, 2005). However, these studies are often carried out in small, nearly two-dimensional, Petri dish arenas where the study organism is unnaturally offered host material thereby greatly reducing searching behaviors and eliminating ecological influences on host searching and utilization (Messing et al., 2006). Often such studies are adequate for determining the physiological host range of an ABCA, but such tests have the potential to overestimate the ecological host range of a natural enemy and therefore may be an unreliable estimator of risk in natural conditions (van Lenteren et al., 2006). Few of the newly proposed testing strategies have been widely implemented and adopted, and subsequently paired with post-release information to provide insight to the reliability of the estimated host-range provided by a particular testing strategy (Barratt et al., 1997; Barratt et al., 2006; van Lenteren et al., 2006; Van Driesche and Reardon, 2004).

The glassy-winged sharpshooter (GWSS), *Homalodisca vitripennis* (Germar) (=*Homalodisca coagulata* [Say] [Takiya et al., 2006]) (Hemiptera: Cicadellidae) classical biological control program in California is a model system that can be used to retroactively test proposed risk assessment methodologies and subsequently compare test results with post-release information collected from field surveys of native non-target species. GWSS is native to the southeastern United States and northeastern Mexico (Turner and Pollard, 1959) and was first collected in California in 1990 (Sorensen and Gill, 1996). As an efficient vector of the bacterium *Xylella fastidiosa* Wells et al., the causal agent of the incurable Pierce's Disease of grapevine, oleander and almond leaf scorches, and other xylem-related diseases (Blua et al., 1999; Redak et al., 2004), area-wide management programs incorporating releases of exotic egg-parasitoids for control of GWSS have been implemented (CDFA, 2003). Five exotic parasitoids have been mass reared and released for control of GWSS since 2001 (CDFA, 2005), including *Gonatocerus ashmeadi* Girault and *G. fasciatus* Girault (Hymenoptera: Mymaridae). *Gonatocerus ashmeadi* is a bi-parental solitary egg-parasitoid believed to have been self-introduced into California on imported GWSS eggs (Vickerman et al., 2004) sometime prior to 1978 (Huber, 1988). Natural populations of *G. ashmeadi* in California have been augmented with mass reared individuals from populations originating in the southeastern USA and northeastern Mexico (CDFA, 2003). *Gonatocerus fasciatus* is a bi-parental gregarious egg-parasitoid capable of producing 2–7 offspring per single GWSS egg (Triapitsyn et al., 2003). *Gonatocerus fasciatus* was mass reared and released in California starting in 2002 from populations originating in southeastern USA (CDFA, 2003).

California has many native cicadellid sharpshooters that are potential non-target hosts for these exotic egg-parasitoids. Native non-target species include, but are not limited to, the smoke-tree sharpshooter (STSS; native congener to GWSS; *Homalodisca liturata* Ball), the blue-green sharpshooter (BGSS; *Graphocephala atropunctata* [Signoret]), and the green sharpshooter (GSS; *Draeculacephala minerva* Ball) (Hemiptera: Cicadellidae). Each cicadellid species listed is a vector of *X. fastidiosa* (Redak et al., 2004). GWSS and STSS both belong to the cicadellid tribe Proconiini; GSS and BGSS belong to the closely related cicadellid tribe Cicadellini (Redak et al., 2004).

The objective of this study was to evaluate no-choice and choice host range testing strategies for two natural enemies being used in an existing biological control program and determine if these laboratory-based results support the results of medium term surveys of the field habitats of selected native non-target species. This research involved the use of relatively small Petri dish test arenas to estimate the physiological host range of the ABCA, coupled with larger-scale entire plant test arenas to simulate a more complex environment to estimate ecological host range by incorporating searching behavior of the parasitoid. These experiments were conducted under no-choice and choice

conditions using native and non-native host plant material to remove parasitoid behavior bias due to host plant cues. Consequently, we examined possible non-target impacts of the self-introduced *Gonatocerus ashmeadi* and the recently introduced *G. fasciatus*, egg-parasitoids of GWSS, on three native California sharpshooters: STSS, BGSS, and GSS. Results from laboratory tests were supplemented with field surveys to determine the invasiveness of *G. ashmeadi* and *G. fasciatus* into the habitats of the three selected native species and subsequent attack rates on these three non-target species should habitat incursion occur by these exotic natural enemies.

## 2. Materials and methods

### 2.1. Study organisms

GWSS is bivoltine capable of producing two generations per year in California (Blua et al., 2001). GWSS eggs are deposited parallel to one another in masses just below the epidermal layer of the host plant leaf surface. As many as 30 eggs may be found in a single mass but typically 8–9 eggs in single egg mass are observed on average (Al-Wahaibi, 2004). Individual GWSS eggs measure approximately 2.5 mm long and 0.53 mm wide (Al-Wahaibi, 2004). GWSS can be found in both coastal and irrigated xeric habitats in California and has an extensive host plant range (Redak et al., 2004). STSS is also bivoltine in California (Blua et al., 2001). Eggs are deposited in a similar manner to GWSS on the undersides of leaves with one to 17 eggs per mass, with 7–8 eggs per mass being typical. Individual STSS eggs are slightly smaller in length than GWSS, measuring on average 2.2 mm long and 0.53 mm wide (Al-Wahaibi, 2004). STSS can be found in both coastal and xeric habitats and also has an extensive host plant range (Al-Wahaibi, 2004). GSS is multivoltine and capable of completing up to three generations per year in California (Purcell and Frazier, 1985). Adult GSS are approximately one-half the length of adult GWSS. Similar to GWSS and STSS, GSS also oviposit eggs side by side in masses just below the epidermal layer of the leaf surface. Egg masses can contain 2–18 eggs (Freitag, 1951) with a mean of around six (Boyd unpublished data). Egg dimensions have not been documented. However, it is likely that egg measurements are close to that of its similar-sized congener *Draeculacepha mollipes* (Say) (Napometh and Nishida, 1972), where an egg, on average, measures 1.35 mm long by 0.25 mm wide (Gibson, 1915); approximately one-half the size of a GWSS egg. Host plants for GSS are typically grass species (Freitag, 1951). BGSS is univoltine in California and adults also are approximately one-half the size of GWSS adults. In direct contrast to GWSS, STSS and GSS, BGSS embeds eggs singly into host plant stem material and seldom utilizes leaves as an oviposition substrate (Severin, 1949; Boyd and Hoddle, 2006). Egg size and shape are unknown and distinguishing characteristics for discerning exact egg locations on the host plant prior to nymphal or adult parasitoid eclosion have not been determined and may not be easily achievable (Boyd and Hoddle, 2006). BGSS has a large host range of mainly herbaceous dicots (Purcell, 1976).

GWSS is an exotic insect in California and will be referred to as the 'target.' STSS, BGSS, and GSS are indigenous to California and will be referred to as the 'non-target' insects in this study.

### 2.2. Insect colonies

Target and non-target sharpshooter colonies were housed in a temperature controlled greenhouse at $26.7 \pm 0.6$ °C, $24.8 \pm 13.4$ % RH, and 16:8 L/D in multiple $75 \times 75 \times 75$ cm cages. Greenhouse populations were supplemented with field collected insects each year. STSS were maintained on Eureka lemon (*Citrus limon* [L.] Burm.f. cv. 'Eureka'; Sapindales: Rutaceae) and jojoba (*Simmondsia chinensis* (Link) Schneid.; Euphorbiales: Simmondsiaceae). BGSS colonies were maintained on sweet basil (*Ocimum basilicum* L.; Lamiales: Lamiaceae) and wild grape (*Vitis girdiana* Munson; Rhamnales: Vitaceae). GSS were maintained on milo (*Sorghum bicolor* L. Moench; Cyperales: Poaceae) and rescuegrass (*Bromus catharticus* Vahl.; Cyperales: Poaceae). GWSS colonies were maintained on all of the above plants (Table 1). Eggs and host plants containing eggs were harvested daily to ensure uniform egg age for experiments. Eggs not utilized immediately for experiments were sealed in a plastic bag with a moistened paper towel and stored in a 15 °C temperature cabinet for up to 48 h. Because of daily egg production fluctuations in the colonies, this conservation procedure was necessary to ensure enough eggs were available for experiments. All host eggs utilized in laboratory experiments and parasitoid colonies were 24–72 h old to ensure optimal age for parasitism by *G. ashmeadi* and *G. fasciatus* (Irvin and Hoddle, 2005).

Parasitoid cultures were maintained in dual 40 dram plastic ventilated vials in a temperature controlled room at $25.8 \pm 0.5$ °C, $27.0 \pm 5.2$ % RH, and 16:8 L/D (see Irvin and Hoddle, 2005; for description of dual rearing vials). *Gonatocerus ashmeadi* and *G. fasciatus* colonies were maintained on GWSS host eggs, age 24–72 h, on Eureka lemon. Parasitized egg masses were checked daily for emergence; all newly emerged male and female parasitoids were gently aspirated into 40 dram vials and provided a 50:50 honey-water food source to maximize fecundity and longevity (Irvin and Hoddle, 2007). Female parasitoids used in the experiments were single, naive, one to two days old, mated, and honey-water fed.

### 2.3. Host plants

Host plants used in the host specificity experiments included all plants listed above and in Table 1. Experimental plants were grown in a temperature controlled greenhouse at $26.7 \pm 0.6$ °C and 16:8 L/D in cylindrical black plastic $11 \times 15$ cm pots. Eureka lemon trees were grown in $10 \times 10 \times 20$ cm black plastic seedling sleeves. All plants

Table 1
Host plants used to maintain target and non-target sharpshooter colonies and utilized in no-choice and choice experiments

| Sharpshooter colony | Native or naturalized host plant | Exotic host plant |
|---|---|---|
| STSS[a] | Jojoba (*Simmondsia chinensis* (Link) Schneid.; Euphorbiales: Simmondsiaceae) | Eureka lemon (*Citrus limon* (L.) Burm.f. cv. 'Eureka'; Sapindales: Rutaceae) |
| BGSS[b] | Wild grape (*Vitis girdiana* Munson; Rhamnales: Vitaceae) | Sweet basil (*Ocimum basilicum* L.; Lamiales: Lamiaceae) |
| GSS[c] | Rescuegrass (*Bromus catharticus* Vahl.; Cyperales: Poaceae) | Milo (*Sorghum bicolor* L. Moench; Cyperales: Poaceae) |
| GWSS[d] | All of the above | All of the above |

[a] Smoke-tree sharpshooter, *Homalodisca liturata*.
[b] Blue-green sharpshooter, *Graphocephala atropunctata*.
[c] Green sharpshooter, *Draeculacephala minerva*.
[d] Glassy-winged sharpshooter, *Homalodisca vitripennis*.

were fertilized once a week, except Eureka lemon, which was fertilized once every three mo. Plants were fertilized with Miracle-Gro® All Purpose Plant Food (15-30-15) (Scotts Miracle-Gro Products, Inc., Marysville, OH 43041), except grasses and lemons, which were fertilized with Miracle-Gro® Water Soluble Lawn Food (36-6-6 plus iron) and Miracle-Gro® Shake'n Feed® Continuous Release Citrus, Avocado, & Mango Plant Food (13-7-13 plus micronutrients), respectively. All experimental plants were ≈30 cm in height and leaf area was standardized within each plant species via pruning using visual estimates. Plants were randomly selected from nursery stock for use in all experiments.

### 2.4. Host specificity experiments

#### 2.4.1. No-choice and choice experiments

Host specificity tests were conducted with *G. ashmeadi* and *G. fasciatus* on non-target BGSS, GSS, and STSS eggs using target GWSS eggs as a control in individually paired comparisons. No-choice and choice experiments were conducted at two scales, micro- and macro-scales, to estimate physiological and ecological host range, respectively. Micro-scale no-choice and choice experiments consisted of clipped plant material containing host eggs, placed egg side up in a parafilm-sealed $100 \times 15$ mm (118 $cm^3$) petri dish provisioned with moistened filter paper. Macro-scale no-choice tests consisted of an entire plant contained within a clear three liter (2500 $cm^3$) plastic bottle cage (Boyd et al., 2007). Choice tests at the macro-scale were conducted within a $55 \times 45 \times 45$ cm (111,375 $cm^3$) clear acrylic cage, large enough to accommodate two entire plants. In all macro-scale tests, the open top of the pot was covered with stretched parafilm to cover the potting soil but allowing the plant stem to protrude. The stretched parafilm prevented moisture loss, reduced accidental mortality of parasitoids, and facilitated parasitoid recovery. Each experimental test was conducted utilizing two different host plants for each of the sharpshooters examined. We incorporated at least one native or naturalized host plant for each sharpshooter species examined (Table 1). For each sharpshooter and host plant combination tested, GWSS also was tested on the same host plant. No micro-scale tests were conducted for BGSS and GWSS comparisons on wild grape or basil due to an inability to discern singly oviposited host eggs cryptically embedded by BGSS in host plant stems. Additionally, no micro or macro-scale choice tests were conducted for GSS and GWSS eggs on milo due to low egg production and phenological differences in oviposition times by these two species that made it extremely difficult to have eggs of both species simultaneously available for experiments.

In no-choice tests, each experimental parasitoid was supplied ≈40 eggs of the selected test species for oviposition. BGSS eggs were not discernable in host plant material, therefore *a priori* counts of eggs presented to parasitoids in experimental arenas could not be determined. For choice tests, equal numbers (≈20 eggs each; <72 h of age) of target and non-target eggs on separate pieces of the same species of host plant were simultaneously presented to the test parasitoid. In all tests, the parasitoid was provisioned with a 50% honey-water streak ($\sim 2 \times 60$ mm) across the top of the petri dish or acrylic cage, or saturated cotton bung in the bottle cages. Parasitoids were allowed 24 h to parasitize sharpshooter eggs before removal from the test arena.

All tests were conducted under laboratory conditions ($25.8 \pm 0.5$ °C, $27.0 \pm 5.2$% RH, and 16:8 L/D) and experimental eggs were held until cicadellid nymphs or parasitoids had enclosed or until 4 wk had passed. All cicadellid eggs were examined under a Leica MZ12 stereomicroscope at 10× zoom (Leica Microsystems Inc., Bannockburn, IL). Parameters measured for GWSS, STSS, and GSS eggs were: (1) parasite emerged; (2) egg parasitized but parasitoid did not emerge; (3) non-emerged cicadellid nymph; (4) emerged nymph, and (5) non-emerged unidentifiable entity. Emerged and non-emerged parasitized eggs were summed and used to calculate proportion parasitism for each cicadellid species. Eggs of BGSS were not discernable, therefore only emerged insects were tabulated and used in parasitism estimates.

#### 2.4.2. Reciprocal tests

To discern whether *G. ashmeadi* and *G. fasciatus* were competent at time of testing, a subsample of parasitoids exposed to GSS and BGSS non-target eggs for 24 h, were

subsequently tested on target host eggs (~40 eggs) for 24 h immediately following the non-target exposure period.

### 2.5. Surveys of native cicadellid habitats for incursion and non-target impacts by Gonatocerus spp. parasitoids

Native cicadellid habitats were sampled with sticky traps, malaise traps, sentinel plants, and collection of host plant material to determine if habitat incursion occurred and if native non-target species were attacked by either *G. ashmeadi* or *G. fasciatus*.

#### 2.5.1. Field sites

Multiple field sites were selected for each of the native non-target cicadellid species. For BGSS three field sites were selected. Two of the sites were west of Temecula, Riverside Co., California, a major wine grape growing region in southern California, in the Sandia Creek area. Both of these sites were bordered by a creek with year-round surface water. Site one was in a shaded ravine, ~30 m deep (hereafter referred to as 'Temecula-creek'). The second site was 3.9 km southwest of the Temecula-creek site along a roadside (hereafter referred to as 'Temecula-road'). Initial observations of BGSS at these field sites revealed the greatest visual counts of adult feeding and copulation, nymphal feeding, and exuviae on wild grape (*Vitis girdiana*), stinging nettle (*Urtica* sp.; Urticales: Urticaceae), mugwort (*Artemesia douglasiana* Bess. ex Hook.; Asterales: Asteraceae), and cocklebur (*Xanthium strumarium* L.; Asterales: Asteraceae), all known host plants for BGSS (Purcell, 1976; Boyd and Hoddle, 2006). Therefore, these plants were sampled for BGSS eggs and parasitoids at these two sites. The third BGSS site was located in Laguna Beach, Orange Co., California. Here, BGSS were found to feed and reproduce on two ornamental host plants, *Rhaphiolepis indica* (L.) Lindl. ex. Ker Gawl. (Rosales: Rosaceae) and *Metrosideros excelsus* Soland ex. Gaertn. (Myrtales: Myrtaceae). These ornamental plants were sampled for BGSS eggs and parasitoids.

For GSS, three sites were surveyed. The first was west of Temecula, also in the Sandia Creek area with nutsedge (*Cyperus esculentus* L.; Cyperales: Cyperaceae), and dallisgrass (*Paspalum dilatatum* Poir.; Cyperales: Cyperaceae) being the dominant understory plants with greatest visual counts by GSS adults, nymphs, and eggs. This was a creek-side site bordered by wild grape. The second GSS site was in Riverside, Riverside Co., California, at the University of California Agricultural Operations (UCR AgOps) in a water waste-way dominated by bermudagrass (*Cynodon dactylon* (L.) Pers.; Cyperales: Poaceae), dallisgrass, and puncturevine (*Tribulus terrestris* L.; Sapindales: Zygophyllaceae). The third GSS site was in Coachella (Riverside Co., California). This site was in the understory of a date (*Pheonix dactylifera* L.; Arecales: Arecaceae) plantation consisting primarily of rescuegrass, annual rabbitsfoot grass (*Polypogon monspeliensis* (L.) Desf.; Cyperales: Poaceae), and bermudagrass as the dominant vegetation.

For STSS, two sites were surveyed. The first was in Riverside at UCR AgOps in plots of jojoba and rough lemon (*Citrus jambhiri* Lush.; Sapindales: Rutaceae; the same two sites utilized by Al-Wahaibi, 2004) bordered by plots of various citrus varieties (*Citrus* sp.; Sapindales: Rutaceae), willow (*Salix* sp.; Salicales: Salicaceae), avocado (*Persea americana*; Laurales: Lauraceae), Brazilian pepper tree (*Schinus terebinthifolius* Raddi; Sapindales: Anacardiaceae) and fig trees (*Ficus* sp.; Urticales: Moraceae). The second site was located in the Coachella Valley near La Quinta (Riverside Co., California). This site was a xeric wash area dominated by native California desert plants including smoke-tree (*Psorothamnus spinosus* (Gray); Fabales: Fabaceae), palo verde (*Parkinsonia* sp.; Fabales: Fabaceae), and various sage brushes (*Artemisia* sp.; Asterales: Asteraceae). This xeric desert site was bordered by a field of irrigated table grapes.

#### 2.5.2. Trapping

For BGSS, a total of 12 yellow double-sided sticky traps (11 × 15 cm), were placed at the two Temecula field sites (six at each site) to monitor BGSS adult and parasitoid flight activity. Traps were deployed on 9 January 2004 and collected and replaced with new traps every two week for 2 yr ending on 6 January 2006. Sticky traps were attached with binder clips to the horizontal arm of a 't' shaped wooden stake. Three of these wooden stakes were deployed at each study site. Sticky traps were set up in pairs on the horizontal arm 22 cm apart and the distance between wooden stakes with traps was 7.8 m. Sticky traps were set up in pairs on stakes 22 cm apart and 14.8 m between stakes. Sticky traps for the 21 January 2005 sample period were destroyed due to severe flooding at the Temecula-creek study site which washed away equipment. Sticky traps were re-deployed on 9 March 2005 after storm and flood activity ceased.

For GSS, a malaise trap was deployed on 4 March 2005 at the AgOps field site. Insects were trapped in 70% ethanol and trap samples were collected every two weeks until 3 November 2006. Trap collections were removed from the field and stored in a freezer until the samples could be sorted for presence of the exotic GWSS parasitoids. Malaise trap samples were not effective in trapping GSS, so trapping was supplemented with sweep net samples consisting of 0.5 h of continuous sweeps conducted at the same 2-week trap collection intervals. Sweep net samples were released immediately following tabulation of GSS captured.

For STSS, a total of 10 yellow double-sided sticky traps (15 × 15 cm), were placed at the AgOps field site to confirm presence of STSS and parasitoids. Five sticky cards each were placed on stakes 7.3 m apart and 15 cm above the ground in two linear transects; one transect was located in the center of a rough lemon plot adjacent to the jojoba plot and the other located along the edge of the jojoba plot. Traps were deployed on 10 February 2006 and collected and replaced with new traps every 2 wk until 19 May 2006.

All sticky traps used at BGSS and STSS field sites were sealed in clear plastic storage bags and deposited into a freezer until counts of native cicadellid species and exotic GWSS parasitoids could be tabulated.

#### 2.5.3. Collection of non-target cicadellid eggs for parasitoid rearing

For BGSS, samples of wild grape, cocklebur, and stinging nettle containing BGSS eggs were collected once on 5 August 2003. Additional wild grape cane samples containing BGSS eggs were collected every two weeks, for 2 yr starting in April and ceasing in November of 2004 and 2005, respectively (Boyd and Hoddle, 2006). Plant samples of *M. excelsus* and *R. indica* from Laguna Beach containing BGSS eggs were collected on 20 May 2004 and 12 April, 28 April, and 3 May 2005. All BGSS eggs were held in the laboratory for nymph and parasitoid emergence.

For GSS egg collection, host plants were visually surveyed at field sites and eggs were collected on days they were located. Due to their rarity, eggs were collected from rescuegrass and rabbitsfoot grass at the Coachella site on 27 March and 3 April 2006, bermudagrass from AgOps on 19 May 2005, and dallisgrass and nutsedge from Temecula on 30 June 2005 and 21 June 2006. Field collected eggs were returned to the laboratory and held for nymph and parasitoid emergence.

STSS eggs were collected from smoke-tree plants on 5 and 20 May 2005 from La Quinta. STSS eggs were extensively sampled from jojoba and rough lemon plots for parasitism at UCR AgOps in 2002 and 2003 as part of a previous study (Al-Wahaibi, 2004) and these comprehensive data sets were used in analyses.

All GSS and STSS eggs were placed in petri dishes provisioned with moistened filter paper and held at laboratory conditions for up to 45 d or until eclosion of nymphs or parasitoids occurred.

#### 2.5.4. Sentinel eggs

To examine if parasitism by exotic GWSS parasitoids was occurring in non-target natural habitats, sentinel plants with non-target sharpshooter eggs were deployed at all the study sites. The deployed host plants were exposed to non-target insect colonies for 3 d to allow for oviposition. Plants were then removed from the colonies and transported to their respective non-target field sites and remained for 3 d. For BGSS, three sweet-basil, one chrysanthemum, and two wild grape plants were utilized in 2003 and 2004. Plants were deployed on 30 August and 1 August and returned to the lab on 2 September and 4 August in 2003 and 2004, respectively. Three milo plants containing GSS eggs were deployed on 16 March and returned to the lab on 19 March 2006. No sentinel eggs were utilized for STSS because high numbers were previously collected by Al-Wahaibi (2004) at multiple field sites. After the exposure period, deployed plants were visually inspected, cleaned of all larval and adult insects, and placed separately into clear acrylic cages in the laboratory. Plants were observed daily for 10 week and numbers of emerging non-target nymphs and parasitoids were recorded.

### 2.6. Statistical analysis

Choice and no-choice testing results were not normally distributed, and required nonparametric analyses. For no-choice tests, a non-parametric independent samples *t*-test was performed on the ranked proportion parasitism of non-target and target eggs by parasitoid, by host plant, and by testing scale using the Wilcoxon–Mann–Whitney test ($\alpha = 0.05$) (PROC NPAR1WAY WILCOXON, SAS Institute 1999). Exact two-tailed *P*-values were calculated (PROC NPAR1WAY WILCOXON, SAS Institute, 1999) for comparisons with $n < 39$. *Z* approximation *P*-values were utilized for comparisons where $n \geqslant 39$ because of failure of convergence of exact Wilcoxon values due to computer memory limitations. To determine if parasitism rates differed by host plant for each host egg, this test was also performed for no-choice test results of ranked proportion parasitism on each host plant, by non-target or target eggs, by parasitoid species, and by testing scale.

For choice tests, a non-parametric paired samples *t*-test was performed on the ranked proportion parasitism of non-target and target eggs by parasitoid, by host plant, and by testing scale using the Wilcoxon Signed Ranks test ($\alpha = 0.05$) (PROC UNIVARIATE, SAS Institute 1999). In all non-parametric analyses, average ranks were used in the case of ties (Conover, 1999). All averages and errors presented herein are based on non-ranked data.

## 3. Results

### 3.1. Host specificity analysis – no-choice tests

#### 3.1.1. Micro-scale no-choice tests with G. ashmeadi

Ranked proportion parasitism of STSS and GWSS eggs by *G. ashmeadi* on Eureka lemon was found to be significantly different ($Z = -2.1406$, $P \approx 0.0323$) with a higher proportion of parasitism of STSS eggs being observed (Table 2). However the ranked proportion parasitism of STSS and GWSS eggs by *G. ashmeadi* on jojoba was not statistically different. *Gonatocerus ashmeadi* did not parasitize GSS on rescuegrass, but did parasitize GWSS eggs on rescuegrass. *Gonatocerus ashmeadi* did not parasitize GSS on milo, but did parasitize GWSS eggs on milo (Table 2).

When holding sharpshooter egg-type constant, then comparing between host plants, the ranked proportion parasitism of STSS eggs on Eureka lemon and jojoba by *G. ashmeadi* were not different ($Z = 0.9665$, $P \approx 0.3338$) (Fig. 1). Ranked proportion parasitism of GWSS eggs on Eureka lemon and jojoba also were not significantly different ($Z = 0.3058$, $P \approx 0.7597$). Finally, ranked proportion parasitism of GWSS eggs on rescuegrass and milo were not different ($Z = 0.5324$, $P \approx 0.5944$). *Gonatocerus ashmeadi* did not parasitize GSS eggs on either rescuegrass or milo, thus no comparison was made.

Table 2
Results of no-choice micro-scale host specificity experiments

| Plant host | Non-target eggs | | | | Target eggs | | | *P* value[a] |
|---|---|---|---|---|---|---|---|---|
| | Species | (*n*) | Mean # eggs (±SE)/rep. | Mean proportion parasitism (±SE) | (*n*) | Mean # eggs (±SE)/rep. | Mean proportion parasitism (±SE) | |
| *G. ashmeadi* | | | | | | | | |
| Lemon | STSS[c] | 38 | 34.5 ± 1.6 | 0.70 ± 0.06 | 38 | 37.6 ± 1.2 | 0.52 ± 0.07 | ≈**0.0323** |
| Jojoba | STSS | 47 | 37.7 ± 1.4 | 0.69 ± 0.05 | 19 | 36.8 ± 1.3 | 0.52 ± 0.10 | ≈0.4637 |
| Grape | BGSS[d] | —[b] | — | — | 2 | 25.5 ± 8.5 | 0.35 ± 0.50 | — |
| Basil | BGSS | — | — | — | 3 | 14.7 ± 5.2 | 0.93 ± 0.06 | — |
| Milo | GSS[e] | 4 | 17.0 ± 4.4 | 0.00 | 14 | 30.8 ± 2.8 | 0.26 ± 0.10 | — |
| Rescuegrass | GSS | 5 | 15.0 ± 1.7 | 0.00 | 3 | 21.7 ± 2.8 | 0.43 ± 0.25 | — |
| *G. fasciatus* | | | | | | | | |
| Lemon | STSS | 26 | 36.1 ± 1.5 | 0.44 ± 0.07 | 89 | 39.7 ± 0.3 | 0.21 ± 0.03 | ≈**0.0008** |
| Jojoba | STSS | 19 | 39.8 ± 0.5 | 0.45 ± 0.07 | 54 | 39.0 ± 1.1 | 0.34 ± 0.05 | ≈0.1444 |
| Grape | BGSS | — | — | — | — | — | — | — |
| Basil | BGSS | — | — | — | — | — | — | — |
| Milo | GSS | 4 | 16.0 ± 2.9 | 0.00 | 12 | 35.1 ± 2.6 | 0.19 ± 0.06 | — |
| Rescuegrass | GSS | 22 | 13.0 ± 1.1 | 0.07 ± 0.03 | 12 | 21.5 ± 2.0 | 0.16 ± 0.07 | **0.0442** |

Physiological host range estimates for *Gonatocerus ashmeadi* and *G. fasciatus* on four sharpshooter host species reared on California native and non-native plants.

[a] Wilcoxon–Mann–Whitney test; significant differences indicated in bold typeface.

[b] Test or analysis not conducted.

[c] Smoke-tree sharpshooter, *Homalodisca liturata*.

[d] Blue-green sharpshooter, *Graphocephala atropunctata*.

[e] Green sharpshooter, *Draeculacephala minerva*.

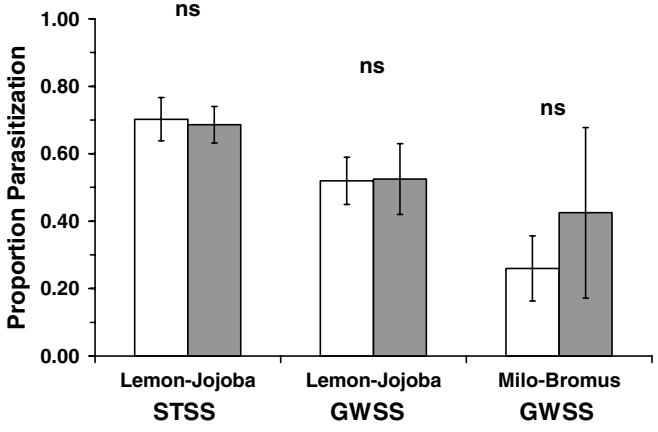


Fig. 1. Comparison of native and non-native California host plant proportion parasitization of *Homalodisca liturata* and *H. vitripennis* (the smoke-tree sharpshooter [STSS] and glassy-winged sharpshooter [GWSS]) eggs by *Gonatocerus ashmeadi* in no-choice micro-scale host specificity tests. See Table 2 for sample size. (Wilcoxon–Mann–Whitney test, ns, not statistically significant, ns*, significant at $P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$.).

### 3.1.2. Micro-scale no-choice tests with G. fasciatus

Ranked proportion parasitism of STSS and GWSS eggs by *G. fasciatus* on Eureka lemon was found to be significantly different ($Z = 3.3686$, $P \approx 0.0008$) with a higher proportion of parasitism of STSS eggs being observed (Table 2). Ranked proportion parasitism of STSS and GWSS eggs by *G. fasciatus* on jojoba were not different. Ranked proportion parasitism of GSS and GWSS eggs by *G. fasciatus* on rescuegrass were significantly different ($Z = 1.9832$, $P = 0.0442$) with a higher proportion parasitism of GWSS eggs. On milo, *G. fasciatus* did not parasitize GSS eggs, but did parasitize GWSS eggs (Table 2).

When holding sharpshooter egg-type constant, then comparing between host plants, the ranked proportion parasitism of STSS eggs on Eureka lemon and jojoba by *G. fasciatus* were not significantly different ($Z = 0.0807$, $P \approx 0.9357$) (Fig. 2). However the ranked proportion parasitism of GWSS eggs on Eureka lemon and jojoba were different ($Z = 2.4706$, $P \approx 0.0135$) (Fig. 2). *Gonatocerus*

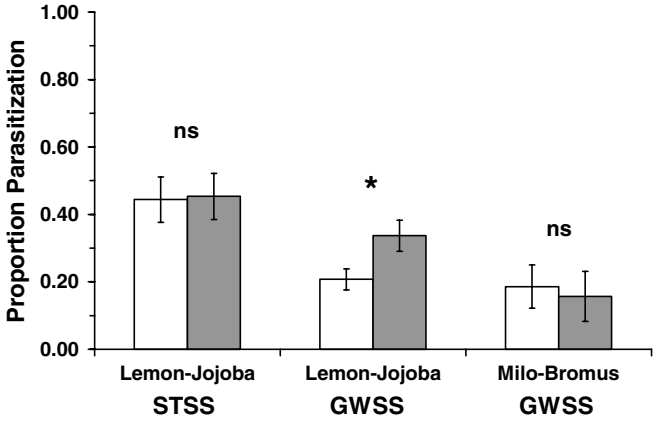


Fig. 2. Comparison of native and non-native California host plant proportion parasitization of *Homalodisca liturata* and *H. vitripennis* (the smoke-tree sharpshooter [STSS] and glassy-winged sharpshooter [GWSS]) eggs by *Gonatocerus fasciatus* in no-choice micro-scale host specificity tests. See Table 2 for sample size. (Wilcoxon–Mann–Whitney test, ns, not statistically significant, ns*, significant at $P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$.).

*fasciatus* did not parasitize GSS eggs on milo, thus no comparison was made with rescuegrass. Ranked proportion parasitism of GWSS eggs on rescuegrass and milo were not different ($Z = 0.3339$, $P \approx 0.7384$).

### 3.1.3. Macro-scale no-choice tests with G. ashmeadi

Ranked proportion parasitism of STSS and GWSS eggs by *G. ashmeadi* on Eureka lemon was different at the $P = 0.10$ level ($Z = -1.8411$, $P \approx 0.0656$) (Table 3). However, ranked proportion parasitism of STSS and GWSS eggs by *G. ashmeadi* on jojoba were not different. *Gonatocerus ashmeadi* did not parasitize GSS eggs; however, no GWSS controls were run for rescuegrass as GWSS eggs were unavailable at the time these studies were run. *Gonatocerus ashmeadi* did not parasitize GSS on milo, but did parasitize GWSS eggs on this host plant (Table 3); this parasitoid did not parasitize BGSS on wild grape, but did parasitize GWSS eggs on this host plant; and *G. ashmeadi* did not parasitize BGSS on basil, but did parasitize GWSS eggs on this host plant (Table 3).

When holding sharpshooter egg-type constant, and then comparing between host plants, the ranked proportion parasitism of STSS eggs on Eureka lemon and jojoba by *G. ashmeadi* was different at the $P = 0.10$ level ($Z = 1.9529$, $P \approx 0.0508$) (Fig. 3). The ranked proportion parasitism of GWSS eggs on Eureka lemon and jojoba also were significantly different ($Z = 2.9165$, $P \approx 0.0035$) with a higher proportion parasitism of GWSS eggs on jojoba being observed (Fig. 3). *Gonatocerus ashmeadi* did not parasitize GSS eggs on either rescuegrass or milo, thus no comparisons were made. No GWSS macro-scale no-choice tests were conducted with rescuegrass, therefore no comparisons could be made with milo. *Gonatocerus ashmeadi* did not parasitize BGSS eggs on either wild grape or basil. Ranked proportion parasitism of GWSS eggs on wild grape and basil were not different ($Z = 0.8485$, $P = 0.3943$).

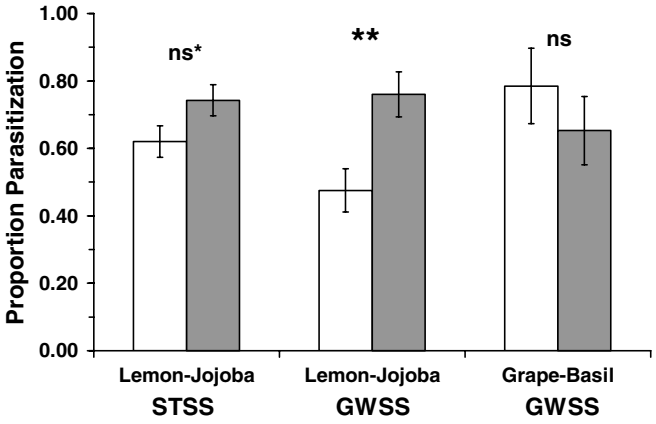


Fig. 3. Comparison of native and non-native California host plant proportion parasitization of *Homalodisca liturata* and *H. vitripennis* (the smoke-tree sharpshooter [STSS] and glassy-winged sharpshooter [GWSS]) eggs by *Gonatocerus ashmeadi* in no-choice macro-scale host specificity tests. See Table 3 for sample size. (Wilcoxon–Mann–Whitney test, ns, not statistically significant, ns*, significant at $P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$.).

### 3.1.4. Macro-scale no-choice tests with G. fasciatus

Ranked proportion parasitism of STSS and GWSS eggs by *G. fasciatus* on Eureka lemon were not significantly different (Table 3). Ranked proportion parasitism of STSS

Table 3
Results of no-choice macro-scale host specificity experiments

| Plant host | Non-target eggs | | | | Target eggs | | | *P* value[a] |
|---|---|---|---|---|---|---|---|---|
| | Species | (*n*) | Mean # eggs (±SE)/rep. | Mean proportion parasitism (±SE) | (*n*) | Mean # eggs (±SE)/rep. | Mean proportion parasitism (±SE) | |
| *G. ashmeadi* | | | | | | | | |
| Lemon | STSS[c] | 49 | 33.7 ± 1.1 | 0.62 ± 0.05 | 37 | 39.9 ± 1.1 | 0.48 ± 0.06 | ≈**0.0656** |
| Jojoba | STSS | 45 | 36.4 ± 1.0 | 0.74 ± 0.05 | 21 | 37.7 ± 1.2 | 0.76 ± 0.07 | ≈0.5016 |
| Grape | BGSS[d] | 15 | 8.5 ± 3.7 | 0.00 | 9 | 33.7 ± 3.4 | 0.79 ± 0.11 | —[b] |
| Basil | BGSS | 35 | 7.5 ± 1.7 | 0.00 | 13 | 31.1 ± 1.5 | 0.65 ± 0.10 | — |
| Milo | GSS[e] | 1 | 6 | 0.00 | 2 | 39.0 ± 1.0 | 0.74 ± 0.03 | — |
| Rescuegrass | GSS | 13 | 30.8 ± 5.6 | 0.00 | — | — | — | — |
| *G. fasciatus* | | | | | | | | |
| Lemon | STSS | 30 | 36.6 ± 1.0 | 0.37 ± 0.07 | 34 | 39.8 ± 0.3 | 0.40 ± 0.06 | ≈0.6643 |
| Jojoba | STSS | 28 | 33.3 ± 1.7 | 0.33 ± 0.07 | 64 | 37.9 ± 0.7 | 0.29 ± 0.04 | ≈0.9829 |
| Grape | BGSS | 13 | 2.2 ± 0.6 | 0.00 | 4 | 40.5 ± 2.6 | 0.40 ± 0.17 | — |
| Basil | BGSS | 12 | 3.8 ± 1.2 | 0.00 | 16 | 23.6 ± 2.1 | 0.47 ± 0.10 | — |
| Milo | GSS | 2 | 9.0 ± 4.0 | 0.00 | 6 | 34.8 ± 3.9 | 0.97 ± 0.02 | — |
| Rescuegrass | GSS | 30 | 18.7 ± 2.9 | 0.00 | 6 | 22.2 ± 2.2 | 0.44 ± 0.12 | — |

Ecological host range estimates for *Gonatocerus ashmeadi* and *G. fasciatus* on four sharpshooter host species reared on California native and non-native plants.

[a] Wilcoxon–Mann–Whitney test; significant differences indicated in bold typeface.

[b] Test or analysis not conducted.

[c] Smoke-tree sharpshooter, *Homalodisca liturata*.

[d] Blue-green sharpshooter, *Graphocephala atropunctata*.

[e] Green sharpshooter, *Draeculacephala minerva*.

and GWSS eggs by *G. fasciatus* on jojoba were not significantly different. *Gonatocerus fasciatus* did not parasitize GSS eggs on rescuegrass, but did parasitize GWSS eggs on this host plant; this parasitoid did not parasitize GSS eggs on milo, but did parasitize GWSS eggs on this host plant; it did not parasitize BGSS eggs on wild grape, but did parasitize GWSS eggs on this host plant, and *G. fasciatus* did not parasitize BGSS eggs on basil, but did parasitize GWSS eggs on this host plant (Table 3).

When holding sharpshooter egg-type constant, then comparing between host plants, the ranked proportion parasitism of STSS eggs on Eureka lemon and jojoba by *G. fasciatus* were not different ($Z = -0.3792$, $P \approx 0.7045$) (Fig. 4). Ranked proportion parasitism of GWSS eggs on Eureka lemon and jojoba were not significantly different ($Z = 1.3385$, $P \approx 0.1807$) (Fig. 4). *Gonatocerus fasciatus* did not parasitize GSS eggs on either rescuegrass or milo, thus no comparisons were made. Ranked proportion parasitism of GWSS eggs on rescuegrass and milo were significantly different ($Z = -2.8526$, $P = 0.0022$) with higher proportion parasitism of GWSS eggs on milo being observed. *Gonatocerus fasciatus* did not parasitize BGSS eggs on either wild grape or basil, thus no comparison was made. Ranked proportion parasitism of GWSS eggs on wild grape and basil were not different ($Z = -0.1439$, $P = 0.8627$).

### 3.1.5. Reciprocal tests for parasitoid competency

Parasitoids utilized in host range experiments were determined to be competent and capable of parasitizing target host eggs after exposure to GSS and BGSS non-target eggs. After exposure to GSS eggs proportion parasitism (±SE) averaged $0.50 \pm 0.29$ for *G. ashmeadi* ($n = 4$) and $0.50 \pm 0.14$ for *G. fasciatus* ($n = 7$) exposed to target eggs. After exposure to BGSS eggs proportion parasitism (±SE) was 0.14 for *G. ashmeadi* ($n = 1$) and averaged $0.38 \pm 0.19$ for *G. fasciatus* ($n = 3$) exposed to target eggs. Reciprocal test replicates were not included if the parasitoid died during the test, therefore replicate numbers are low.

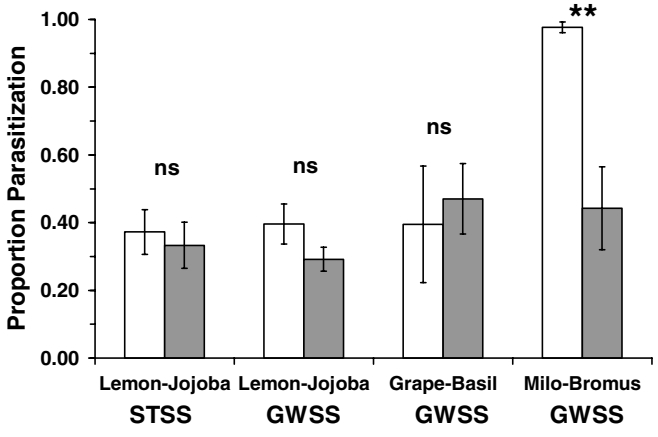


Fig. 4. Comparison of native and non-native California host plant proportion parasitization of *Homalodisca liturata* and *H. vitripennis* (the smoke-tree sharpshooter [STSS] and glassy-winged sharpshooter [GWSS]) eggs by *Gonatocerus fasciatus* in no-choice macro-scale host specificity tests. See Table 3 for sample size. (Wilcoxon–Mann–Whitney test, ns, not statistically significant, ns*, significant at $P < 0.10$, $^{*}P < 0.05$, $^{**}P < 0.01$.).

## 3.2. Host specificity analysis—choice tests

### 3.2.1. Micro-scale choice tests with G. ashmeadi

The ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. ashmeadi* on Eureka lemon were not different (Table 4). However, the ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. ashmeadi* on jojoba were significantly different ($S = -14.0$, $P = 0.0156$), with a higher proportion parasitism of STSS eggs being observed (Table 4). *Gonatocerus ashmeadi* did not parasitize GSS eggs, but did parasitize GWSS eggs on rescuegrass (Table 4). No micro-scale choice tests were conducted with GSS and GWSS eggs on milo because eggs of both test species were not available concurrently. No micro-scale choice tests were conducted with BGSS and GWSS eggs on either host plant.

### 3.2.2. Micro-scale choice tests with G. fasciatus

Ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. fasciatus* on Eureka lemon were not significantly different (Table 4). The ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. fasciatus* on jojoba were different at the $P = 0.10$ level ($S = -25.0$, $P = 0.0833$), with higher proportion parasitism of STSS eggs (Table 4). Ranked proportion parasitism of GSS and GWSS eggs offered simultaneously to *G. fasciatus* on rescuegrass were not different (Table 4). *Gonatocerus fasciatus* was capable of successfully producing two female offspring per GSS egg. However, the female offspring (Voucher No.: UCRC ENT 146580) were missing longitudinal sensilla on the 6th funicular antennal segment, indicative of development on a smaller host (S.V. Triapitsyn, personal communication). Voucher specimens were deposited at the UCR Entomology Research Museum, Riverside, CA, USA. No micro-scale choice tests were conducted with GSS and GWSS eggs on milo because of a lack of concurrent egg availability due to cicadellid egg laying phenology. No micro-scale choice tests were conducted with BGSS and GWSS eggs on either host plant.

### 3.2.3. Macro-scale choice tests with G. ashmeadi

Ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. ashmeadi* on Eureka lemon were not significantly different (Table 5). Unlike the micro-scale choice tests, the ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. ashmeadi* on jojoba were not significantly different. *Gonatocerus ashmeadi* did not parasitize GSS eggs, but did parasitize GWSS eggs on rescuegrass (Table 5). No macro-scale choice tests were conducted with GSS and GWSS eggs on milo. *G. ashmeadi* did not parasitize BGSS eggs on wild grape, but did parasitize GWSS eggs on this host plant; this

Table 4
Results of choice micro-scale host specificity experiments

| Plant host | Species | (*n*) | Non-target eggs | | Target eggs | | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | | | Mean # eggs (±SE)/rep. | Mean proportion parasitism (±SE) | Mean # eggs (±SE)/rep. | Mean Proportion Parasitism (±SE) | |
| *G. ashmeadi* | | | | | | | |
| Lemon | STSS[c] | 48 | 19.7 ± 0.3 | 0.54 ± 0.06 | 19.8 ± 0.3 | 0.56 ± 0.07 | 0.5767 |
| Jojoba | STSS | 23 | 19.5 ± 0.8 | 0.90 ± 0.06 | 19.5 ± 0.8 | 0.80 ± 0.08 | **0.0156** |
| Grape | BGSS[d] | —[b] | — | — | — | — | — |
| Basil | BGSS | — | — | — | — | — | — |
| Milo | GSS[e] | — | — | — | — | — | — |
| Rescuegrass | GSS | 1 | 14 | 0.00 | 14 | 0.21 | — |
| *G. fasciatus* | | | | | | | |
| Lemon | STSS | 78 | 19.2 ± 0.3 | 0.41 ± 0.04 | 19.2 ± 0.3 | 0.36 ± 0.04 | 0.2818 |
| Jojoba | STSS | 21 | 18.8 ± 0.8 | 0.42 ± 0.08 | 19.1 ± 0.8 | 0.32 ± 0.08 | **0.0833** |
| Grape | BGSS | — | — | — | — | — | — |
| Basil | BGSS | — | — | — | — | — | — |
| Milo | GSS | — | — | — | — | — | — |
| Rescuegrass | GSS | 9 | 8.9 ± 1.1 | 0.20 ± 0.12 | 9.6 ± 0.7 | 0.19 ± 0.13 | 1.0000 |

Physiological host range estimates for *Gonatocerus ashmeadi* and *G. fasciatus* on four sharpshooter host species reared on California native and non-native plants.
[a] Wilcoxon Signed Ranks test; significant differences indicated in bold typeface.
[b] Test or analysis not conducted.
[c] Smoke-tree sharpshooter, *Homalodisca liturata*.
[d] Blue-green sharpshooter, *Graphocephala atropunctata*.
[e] Green sharpshooter, *Draeculacephala minerva*.

Table 5
Results of choice macro-scale host specificity experiments

| Plant host | Species | (*n*) | Non-target eggs | | Target eggs | | *P* value[a] |
|---|---|---|---|---|---|---|---|
| | | | Mean # eggs (±SE)/rep. | Mean Proportion Parasitism (±SE) | Mean # eggs (±SE)/rep. | Mean proportion parasitism (±SE) | |
| *G. ashmeadi* | | | | | | | |
| Lemon | STSS[c] | 15 | 34.2 ± 2.0 | 0.54 ± 0.09 | 34.1 ± 2.1 | 0.64 ± 0.09 | 0.3013 |
| Jojoba | STSS | 21 | 33.9 ± 2.3 | 0.39 ± 0.09 | 33.9 ± 2.3 | 0.50 ± 0.10 | 0.2788 |
| Grape | BGSS[d] | 14 | 4.0 ± 1.5 | 0.00 | 20.4 ± 1.9 | 0.38 ± 0.12 | —[b] |
| Basil | BGSS | 3 | 5.0 ± 3.5 | 0.00 | 4.7 ± 0.9 | 1.00 ± 0.00 | — |
| Milo | GSS[e] | — | — | — | — | — | — |
| Rescuegrass | GSS | 6 | 19.2 ± 4.6 | 0.00 | 15.5 ± 3.8 | 0.17 ± 0.17 | — |
| *G. fasciatus* | | | | | | | |
| Lemon | STSS | 40 | 20.3 ± 0.7 | 0.43 ± 0.06 | 20.3 ± 0.7 | 0.37 ± 0.06 | 0.3020 |
| Jojoba | STSS | 17 | 17.5 ± 1.0 | 0.23 ± 0.09 | 17.2 ± 0.9 | 0.28 ± 0.09 | 0.4609 |
| Grape | BGSS | 11 | 3.4 ± 0.9 | 0.00 | 25.5 ± 3.2 | 0.46 ± 0.14 | — |
| Basil | BGSS | 35 | 13.2 ± 3.2 | 0.00 | 25.5 ± 2.7 | 0.36 ± 0.07 | — |
| Milo | GSS | — | — | — | — | — | — |
| Rescuegrass | GSS | 6 | 12.7 ± 2.6 | 0.0079 ± 0.0079 | 14.3 ± 3.7 | 0.0413 ± 0.0327 | 1.0000 |

Ecological host range estimates for *Gonatocerus ashmeadi* and *G. fasciatus* on four sharpshooter host species reared on California native and non-native plants.
[a] Wilcoxon Signed Ranks test.
[b] Test or analysis not conducted.
[c] Smoke-tree sharpshooter, *Homalodisca liturata*.
[d] Blue-green sharpshooter, *Graphocephala atropunctata*.
[e] Green sharpshooter, *Draeculacephala minerva*.

parasitoid did not parasitize BGSS eggs on basil, but did parasitize GWSS eggs on basil (Table 5).

### 3.2.4. Macro-scale choice tests with G. fasciatus

Ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. fasciatus* on Eureka lemon were not significantly different (Table 5). Similar to *G. ashmeadi*, the ranked proportion parasitism of STSS and GWSS eggs offered simultaneously to *G. fasciatus* on jojoba were not significantly different. Ranked proportion parasitism of GSS and GWSS eggs offered simultaneously to *G. fasciatus* on rescuegrass also were not different,

although, one GSS egg was parasitized in one of six replicates (Table 5). *Gonatocerus fasciatus*, similar to the micro-scale choice tests, produced two offspring per GSS egg. No macro-scale choice tests were conducted with GSS and GWSS eggs on milo (Table 5). *Gonatocerus fasciatus* did not parasitize BGSS eggs on wild grape, but did parasitize GWSS eggs on wild grape; this parasitoid did not parasitize BGSS eggs on basil, but did parasitize GWSS eggs on this host plant (Table 5).

### 3.3. Surveys of native cicadellid habitats for incursion and non-target impacts by Gonatocerus spp. parasitoids

#### 3.3.1. Trapping

Sticky card trap catches revealed that *G. ashmeadi* infiltrated BGSS habitat. Ten *G. ashmeadi* were captured in 2004, and nine in 2005. No *G. fasciatus* were trapped in BGSS habitat (Table 6). A total of 3681 and 1579 BGSS adults were trapped in 2004 and 2005, respectively (Boyd and Hoddle, 2006). Target and non-target sharpshooters also were present in the BGSS habitat. One GWSS was captured in 2004, but none in 2005. Two STSS were trapped in 2004, and three in 2005. Five GSS were captured in both 2004 and 2005.

Malaise trap captures revealed that *G. ashmeadi* had infiltrated GSS habitats. A total of 562 *G. ashmeadi* were captured in 2005 and six in 2006. No *G. fasciatus* were trapped in GSS habitat (Table 6). A total of 562 and 223 GSS were captured in sweep samples conducted in 2005 and 2006, respectively. Target and non-target sharpshooters also were present in the GSS habitat. Forty and 36 GWSS were captured in 2005 and 2006, respectively. Three STSS were captured each year in 2005 and 2006.

A total of 107 and 145 *G. ashmeadi* were trapped in rough lemon and jojoba plots, respectively, in 2006. No *G. fasciatus* were trapped in STSS habitat (Table 6). Trap capture confirmed presence of *G. ashmeadi* in STSS habitat. In spring 2006, 50 and 394 STSS, and 19 and 20 GWSS, were captured in rough lemon and jojoba plots, respectively.

#### 3.3.2. Collection of non-target cicadellids eggs for parasitoid rearing

A total of 333 BGSS eggs were sampled from wild grape, 8 from cocklebur, 18 from stinging nettle, 31 from *R. indica* and 15 from *M. excelsus* during the 2003–2005 collection period. (Since BGSS eggs were not distinguishable in plant material, egg counts reported reflect the actual number of emerged BGSS nymphs and indigenous parasitoids combined.) No *G. ashmeadi* or *G. fasciatus* were reared from any BGSS eggs (Table 6).

A total of 104 GSS egg masses from rescuegrass and rabbitsfoot grass at the Coachella site, one from bermudagrass at AgOps, six from dallisgrass and 13 from nutsedge at Temecula were sampled during the 2005–2006 collection period. No *G. ashmeadi* or *G. fasciatus* were reared from any field collected GSS eggs (Table 6).

Seven STSS egg masses (37 eggs) were sampled in spring 2005 from La Quinta. No *G. ashmeadi* or *G. fasciatus* were reared from any of the STSS eggs (Table 6).

#### 3.3.3. Sentinel eggs

A total of 197 and 23 BGSS eggs were deployed into BGSS habitat in 2003 and 2004, respectively. (Egg counts reported reflect the actual number of emerged BGSS nymphs and indigenous parasitoids combined.) No *G. ashmeadi* or *G. fasciatus* were reared from sentinel BGSS eggs (Table 6).

A total of 25 GSS eggs were deployed into GSS habitat in 2006. No *G. ashmeadi* or *G. fasciatus* were reared from sentinel GSS eggs (Table 6).

## 4. Discussion

In the current study, negative controls were not conducted, therefore normal mortality rates of the target and non-target species during the egg stage is uncertain. Some

Table 6
Non-target habitat survey results showing the presence or absence of exotic parasitoids as determined by three different survey techniques

| Non-target sharpshooter | Field site | Trapping | | Egg collection | | Sentinel eggs | |
|---|---|---|---|---|---|---|---|
| | | *G. ashmeadi* | *G. fasciatus* | *G. ashmeadi* | *G. fasciatus* | *G. ashmeadi* | *G. fasciatus* |
| BGSS[a] | Temecula-creek | Yes | No | No | No | No | No |
| | Temecula-road | Yes | No | No | No | No | No |
| | Laguna Beach | — | — | No | No | — | — |
| GSS[b] | Temecula | Yes | No | No | No | No | No |
| | UCR AgOps | Yes | No | No | No | | |
| | Coachella | — | — | No | No | — | — |
| STSS[c] | UCR AgOps | Yes | No | Yes[d] | No[d] | — | — |
| | La Quinta | — | — | No | No | — | — |

Survey method not employed.
[a] Blue-green sharpshooter, *Graphocephala atropunctata*.
[b] Green sharpshooter, *Draeculacephala minerva*.
[c] Smoke-tree sharpshooter, *Homalodisca liturata*.
[d] Following survey results of Al-Wahaibi (2004).

of the non-emerged nymphs and non-emerged unknowns observed in our studies could have been a result of parasitism and not due to normal physiological mortality. Because we removed the non-emerged nymphs and unknowns from our parasitism rates this type of error could have led us to under estimate the non-target impact of the parasitoids since normal mortality was not corrected for. Mortality for these species (both parasitoids and sharpshooters) during these life stages is relatively low within the protected confines of the leaf (E.A.B. personal observations). Al-Wahaibi and Morse (2003) found that field collected GWSS egg hatch rates for GWSS were 83% from lemon and 56% from jojoba, and ~90% for laboratory reared eggs on chrysanthemum at temperatures 25.6 and 31.2 °C. The total number of unknowns for non-target and target species was only 3.2 and 8.0%, respectively, for *G. ashmeadi* and only 5.9 and 9.2%, respectively, for *G. fasciatus* in our studies. Therefore our estimates of percentage parasitism are likely to be accurate.

Experiments at the micro-scale were used to estimate the physiological host range of *G. ashmeadi* and *G. fasciatus*. The results of these experiments indicate that *G. ashmeadi* and *G. fasciatus* are physiologically capable of parasitizing STSS eggs in both choice and no-choice environments. However, GSS eggs were physiologically acceptable only for *G. fasciatus*, and not for *G. ashmeadi*. *Gonatocerus ashmeadi* and *G. fasciatus* did not exploit BGSS eggs for reproduction.

A continuum of complexity to volumes ratios exists in choice test experiments. Complexity increases from the simple two-dimensional Petri dish study to larger macrocosm cages approximating a simplified three-dimensional habitat cage, and finally approaches a limit at the actual scale of the habitat occupied by the non-target species. At some point along this continuum of complexity there will be a confluence between the physiological and ecological host range that could be determined experimentally. It may be beneficial to the emerging science of host-risk analysis for arthropod biological control agents for workers to consider developing and using a complexity:volume index for a natural enemy by which risk assessment results could be better predicted to measure actual risk in nature.

Experiments conducted here at the macro-scale were designed to provide a better estimate of the ecological host range in nature, by incorporating a greater level of complexity to volume ratio. It has been suggested that arthropod host specificity testing has not given sufficient research attention to this possibility (Louda et al., 2003). The results of laboratory macro-scale tests in this study suggest that *G. ashmeadi* and *G. fasciatus* both would have significant non-target impacts on STSS in the field.

Al-Wahaibi (2004) found that *G. ashmeadi* parasitized eggs of STSS on jojoba and rough lemon at UCR AgOps in 2002 and 2003 at rates up to 100% (as predicted from the retroactive studies conducted here). Approximately 26% and 21% of total STSS egg masses collected during peak spring-time oviposition periods were parasitized by *G. ashmeadi* in 2002 and 2003, respectively. In rough lemon, there was a significantly higher presence of *G. ashmeadi* on STSS egg masses (compared to GWSS) over the entirety of Al-Wahaibi's (2004) 2002–2003 survey period. However, in jojoba, there was no indication of bias for *G. ashmeadi* on STSS or GWSS (Al-Wahaibi, 2004). These comprehensive field observations for *G. ashmeadi* correspond to results obtained in the micro- and macro-scale no-choice tests, where proportion parasitism of STSS eggs was significantly higher than that of GWSS eggs on Eureka lemon, but not on jojoba. In summary, when not given a choice of host eggs, testing scale is not a factor in *G. ashmeadi* parasitism rates, but host plant is a significant factor influencing parasitism rates. Alternatively, *G. fasciatus* parasitism rates of STSS eggs were significantly higher than that of GWSS eggs at the micro-scale, but not at the macro-scale for Eureka lemon, and parasitism rates were not different for jojoba at either scale. Therefore, testing scale influences parasitism rates for *G. fasciatus*, but host plant did not in a no-choice arena. These parasitoid congeners behave differently depending on size of the testing arena and the plant species containing experimental host eggs.

Al-Wahaibi's (2004) field results however, do not correspond to our choice test results at either scale for Eureka lemon since there was no difference between *G. ashmeadi* parasitism rates of STSS and GWSS eggs. At the micro-scale, *G. ashmeadi* parasitized significantly more STSS than GWSS eggs on jojoba, but there was no difference at the macro-scale. The former result is in disagreement, but the latter is in agreement with Al-Wahaibi's (2004) field surveys. The exact same trend was observed for *G. fasciatus* at the micro and macro testing scales on both Eureka lemon and jojoba. Therefore, testing scale on different host plants influences parasitism rates of these closely related parasitoids when given a choice of hosts. Phenological differences in GWSS and STSS oviposition periods may lead to different realized rates of parasitism in the field over the course of the year. Thus, in a test where an equal egg choice is offered, the results may correspond only to a discrete period of GWSS and STSS oviposition overlap in the field. Regardless, the results of our tests raise the question of interpreting and comparing a 'snapshot' of laboratory or field results since life history of target and non-target organisms may have significant dynamic temporal fluctuations in the field.

Also, our laboratory testing results indicate that the point of overlap on the continuum from physiological host range estimates in small scale experiments to ecological host range estimates in large scale experiments can be vastly different for closely related parasitoid species. Hence the validity of *a priori* predictions for potential ecological host range extrapolated from simple physiological host range laboratory experiments, which do not include variables as scale and plant species, should be subject to careful interpretation.

It is difficult to ascertain how well the laboratory tests estimated the actual ecological host range of *G. fasciatus* given that few recoveries of this parasitoid have been made in California since large scale releases were first made in 2002 (Al-Wahaibi, 2004; CDFA, 2005) indicating it is likely this parasitoid is not widely established in large numbers. However, recoveries of *G. fasciatus* made by Al-Wahaibi (2004) were only from the target species. It is possible that the complex of indigenous natural enemies present on STSS eggs especially on the native host plant jojoba (Al-Wahaibi, 2004; Huber, 1988) competitively excluded *G. fasciatus* from utilizing STSS eggs in the field. If *G. fasciatus* has widely established in California, it may currently be at densities below detection and it is possible that numbers may increase to detectable levels in the future. If so, results presented here indicate it will likely pose a non-target risk to STSS and possibly GSS. Additionally, *G. ashmeadi* and *G. fasciatus* were not recovered from eggs of STSS or GWSS in xeric habitats (Al-Wahaibi, 2004), which highlights the need for incorporating climate matching into the pre-release assessment of the proposed biological control agent (Hoddle, 2004a).

Differences in parasitism rates of sharpshooter eggs at the micro level no-choice test arenas on different plant species shows the importance of host plant characteristics on the physiological capability of the parasitoids to access host eggs. Results from macro-scale experiments are likely caused by plant architectural differences in a greater volume arena coupled with the ability of the parasitoid to locate potential host eggs on the plants. We would therefore expect that differences observed at the micro-scale should be even greater at the macro-scale. No significant differences in the proportion parasitism of STSS on citrus and jojoba, GWSS on citrus and jojoba, and GWSS on milo and rescuegrass by *G. ashmeadi* in the micro-scale no-choice test comparisons suggest that these plant species present no significant physiological barriers to parasitoids accessing host eggs (Fig. 1). However, higher rates of parasitism on jojoba as compared to Eureka lemon for STSS and GWSS parasitism by *G. ashmeadi* at the macro level may likely be due to increased complexity in plant architecture (Fig. 3). Citrus plants used in these studies had a greater number of overlapping leaves with larger leaf surface area while jojoba had substantially less leaf overlap and smaller leaves, thereby presenting a less complex habitat within which to search for host eggs.

A significant difference in the proportion parasitism between citrus and jojoba host plants by *G. fasciatus* for GWSS eggs but not for STSS eggs at the micro-scale may suggest that GWSS eggs in jojoba are physiologically more acceptable than those in Eureka lemon (Fig. 2). However, there was no difference in the proportion parasitism between Eureka lemon and jojoba for STSS or GWSS egg parasitism at the macro scale. This is the level where one would expect to see an effect it if this difference was exacerbated for GWSS when host plant architecture is incorporated into testing. It is possible that Eureka lemon was an equally favored host for a different unmeasured variable for the gregarious *G. fasciatus*. This parasitoid may have been allocating a greater number of offspring to fewer eggs on Eureka lemon than jojoba, thus resulting in a lower proportion parasitism when overall number of oviposition events may have been equal. The difference between GWSS egg parasitism on milo and rescuegrass is again likely due to plant architecture. Milo had broader and fewer leaf blades compared to rescuegrass which had relatively thinner, but greater number of leaf blades, thereby increasing architectural complexity.

It is likely that host egg size and location in the host plant strongly influenced successful host egg utilization. Since STSS eggs are very similar to GWSS eggs, it was expected that STSS eggs would be utilized readily by parasitoids. GSS eggs, although deposited in the same manner, are approximately half the size of GWSS eggs. Thus size was probably inadequate for development of the solitary *G. ashmeadi*, but the gregarious nature of *G. fasciatus* allowed for development of two parasitoids per GSS egg.

It is not known what indirect impacts parasitism of STSS eggs by *G. ashmeadi* and other exotic *Gonatocerus* spp. released for control of GWSS (Al-Wahaibi, 2004), will have upon indigenous parasitoid fauna of STSS or the population structure and densities of this insect. Relative abundance of this native cicadellid has not been monitored over time to make prior and post-release comparisons. Also, there is evidence that indigenous STSS egg-parasitoids, *Gonatocerus* spp. and *Ufens* spp. (Hymenoptera: Trichogrammatidae), are capable of parasitizing GWSS eggs and that parasitism varies widely depending upon host plant and geographic location (Al-Wahaibi, 2004). Spill over of these native parasitoid populations breeding on GWSS back into native STSS habitat may exacerbate the effect of parasitism by exotic parasitoids on STSS populations that are resident in native habitat. Consequently, it is not known to what extent these indigenous parasitoids might compete with exotic GWSS parasitoids, or if overall populations of these native parasitoids have risen, declined, or stayed the same. The possibility of these indirect effects may warrant further research. Regardless, non-target parasitism by these natural enemies solidifies the notion that risk associated with the release of generalist natural enemies can have detrimental consequences and only highly host specific biological control agents should be selected via proper implementation and interpretation of host specificity tests prior to their release in classical biological control programs (Hoddle and Syrett, 2002; Hoddle, 2004b).

Although parasitoids successfully parasitized non-target eggs and offspring successfully emerged from non-target eggs, the fitness of the offspring may not be as great as conspecifics emerging from target eggs. *Gonatocerus ashmeadi* offspring reared from GWSS host eggs were on average, 12% larger and contained 40% more eggs than those emerging from STSS eggs (Irvin et al., 2005). Even though a similar study has not been conducted for *G. fasciatus*, offspring did not exhibit any external morphological differences

when reared from STSS eggs, but showed readily observable antennal deformations when reared from smaller GSS eggs. Fitness consequences associated with utilization of non-target hosts may directly or indirectly influence the outcome of a classical biological control program and are factors that are not likely to be accurately captured with results from simple no-choice and choice tests.

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