SHOULD NEOCLASSICAL BIOLOGICAL CONTROL AGENTS FROM ARGENTINA BE RELEASED IN CALIFORNIA FOR CONTROL OF THE GLASSY-WINGED SHARPSHOOTER?

Project Leaders:
Mark Hoddle
Department of Entomology
University of California
Riverside, CA 92521
mark.hoddle@ucr.edu

Nic Irvin
Department of Entomology
University of California
Riverside, CA 92521

Cooperator:
Serguei Triapitsyn
Department of Entomology
University of California
Riverside, CA 92521

Reporting Period: The results reported here are from work conducted April 2007 to October 2007.

ABSTRACT
Results from exposing four to eight glassy-winged sharpshooter (GWSS) eggs to one female parasitoid for one h in a 'complex experimental system' showed that parasitism of eggs one-five days of age ranged from 81-97% for *Gonatocerus ashmeadi* (*G. ashmeadi*), whereas, parasitism by *Gonatocerus tuberculifemur* (*G. tuberculifemur*) ranged from 18-66%. Eggs five days of age were less suitable for *G. tuberculifemur* development (resulting in 18% parasitism). When *G. ashmeadi* and *G. tuberculifemur* were presented simultaneously with one GWSS egg mass for one h (complex system) or 15 min (simple system), parasitism by *G. ashmeadi* was consistently 12-54% higher than *G. tuberculifemur* for all egg ages. There was no significant difference in overall percentage parasitism of GWSS eggs between vials containing *G. ashmeadi* only and vials containing both *G. ashmeadi* and *G. tuberculifemur* for both simple and complex systems. This may be attributable to conspecific presence increasing non-ovipositional behavior such as aggressive chasing (up to 1.3% of time) and antennating conspecifics (up to 1.3% of time), thereby reducing time available for oviposition. This may suggest that the addition of *G. tuberculifemur* to the resident parasitoid guild in California may not enhance biological control of GWSS. Finally, in experiments where 50 GWSS eggs were exposed simultaneously to one female *G. ashmeadi* and *G. tuberculifemur* for 24 h or five days, parasitism by *G. ashmeadi* was 44-53% higher than *G. tuberculifemur* for both exposure times. Results from all completed experiments assessing the effect of environmental complexity, egg age, and competition indicate that *G. tuberculifemur* is an inferior competitor for GWSS egg masses when *G. ashmeadi* is present and that the introduction of *G. tuberculifemur* into California may not benefit GWSS biological control unless this species can efficiently exploit an unknown niche where competition with *G. ashmeadi* is likely to be low or non-existent.

INTRODUCTION
G. tuberculifemur is a sharpshooter parasitoid from Argentina that has been regularly imported into the UCR I & Q facility since September 2002 and reared on GWSS egg masses. There is substantial uncertainty about the safety of releasing this agent and whether it would provide additional control of GWSS in California or disrupt the efficacy of the existing parasitoid complex, which has been constructed with natural enemies that have evolved to exploit GWSS in the home range of this pest. The purpose of this work is to ascertain in Quarantine whether this neoclassical biological control agent from Argentina can outperform the dominant GWSS parasitoid in California, *G. ashmeadi*. These data will help guide the decision to release the Argentinean parasitoid from quarantine for liberation and establishment in California.

OBJECTIVES
This research project has five objectives:
1. Ascertain oviposition preferences of *G. ashmeadi* and *G. tuberculifemur* for GWSS egg masses of different ages.
2. Determine the competitiveness of these two parasitoid species simultaneously foraging for GWSS egg masses in complex and simple environments.
3. Compare the functional response of each species attacking GWSS egg masses of different sizes.
4. Compare the mean daily and lifetime reproductive output for each species at 20, 25, and 30^{o}C.
5. Determine mean developmental times for each species at 20, 25, and 30^{o}C.

To date, we have completed Objectives 1-3. Three experiments were conducted to investigate egg age preferences and competitive ability of *G. ashmeadi* and *G. tuberculifemur*. These involved complex and simple systems and an experiment with long host exposure times. Objectives 4 & 5 will be completed in year 2.

RESULTS
Egg age preferences and competitive ability
Complex system:
One mated female *G. ashmeadi* and *G. tuberculifemur* (~24-36 h) was presented simultaneously to one GWSS egg mass (~four-eight eggs) camouflaged amongst four other similar sized lemon leaves in a double ventilated vial. This 'complex system' was replicated 15 times for GWSS eggs aged one, three and five days of age. After 60 minutes exposure to foraging parasitoids, leaves with egg masses were placed into individual Petri dishes, labeled and held at 27^{o}C for emergence of parasitoids and GWSS nymphs. The number of emerged and unemerged males and females of each parasitoid species was

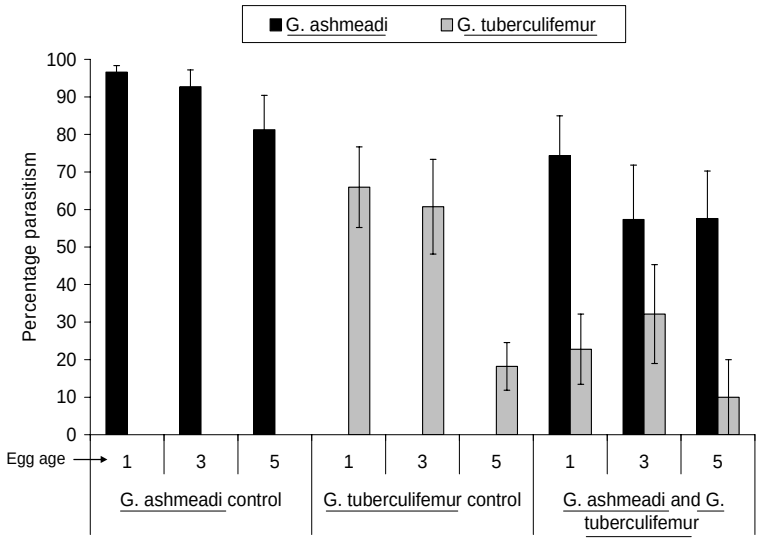

Figure 1. Percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* resulting when GWSS egg masses aged one, three, and five days of age were exposed to parasitoids either alone or in competition with each other.

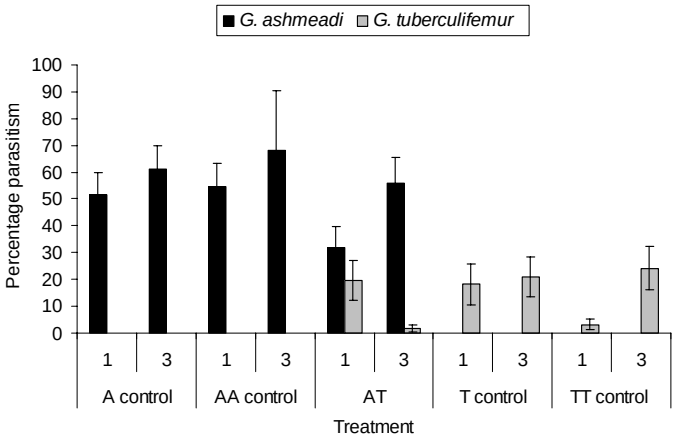

Figure 2. Percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* resulting when GWSS egg masses aged one and three days of age were exposed to parasitoids either alone or with intraspecific or interspecific competition (A – control vial containing one female *G. ashmeadi*; AA = control vial containing two female *G. ashmeadi*; AT = one female *G. ashmeadi* and *G. tuberculifemur*, TT = two female *G. tuberculifemur*; T = one female *G. tuberculifemur*).

recorded. Fifteen control vials containing one female parasitoid were set up for each species. Percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* was calculated as the percentage of total eggs.

Figure 1 shows percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* resulting when GWSS egg masses one, three or five days of age were exposed to three different treatments: (i) *G. ashmeadi* control vials consisting of one female *G. ashmeadi*, (ii) *G. tuberculifemur* control vials consisting of one female *G. tuberculifemur*, and (iii) vials containing one female of both *G. ashmeadi* and *G. tuberculifemur*. Results from the *G. tuberculifemur* control vials show that 60-66% of eggs one and three days of age were successfully parasitized by *G. tuberculifemur* (Figure 1). Eggs five days of age were less suitable for *G. tuberculifemur* development and resulted in just 18% parasitism. In contrast, results from the *G. ashmeadi* control vials showed that *G. ashmeadi* parasitism ranged from 81-97% and there was no significant difference in parasitism between egg ages (Figure 1). This result for *G. ashmeadi* is similar to that observed by Irvin & Hoddle (2005a). The higher rates of parasitism and larger host age range demonstrated by *G. ashmeadi* may indicate that this species may be more competitive than *G. tuberculifemur* and may outcompete it in the field for GWSS egg masses.

Results from vials containing one GWSS egg mass exposed simultaneously to one *G. ashmeadi* and *G. tuberculifemur* in a 'complex experimental system' for one h showed that parasitism by *G. ashmeadi* was consistently 25-51% higher than *G. tuberculifemur* for all three egg ages (Figure 1).

Simple system

One mated female *G. ashmeadi* and *G. tuberculifemur* (~24-36 h) was presented simultaneously to one GWSS egg mass (~four-eight eggs) on a single leaf in a double ventilated vial. This 'simple system' was replicated 15 times for GWSS eggs aged one and three days of age. Egg masses were not camouflaged amongst four other similar sized leaves. Exposure time was 15 mins and each minute the behavior [searching container (SC), searching leaf (SL), searching egg mass (SE), oviposition (O), resting (R), grooming (G), aggressive chasing (C), antennating conspecific (AC), searching egg mass from top side of leaf (SETS), ovipositing from top side of leaf (OTS), feeding (F)] of each female was recorded. Fifteen replicates of two types of control vials were also set up for each species. These contained either one female parasitoid or two female parasitoids of the same species.

Results from vials containing one GWSS egg mass exposed simultaneously to one *G. ashmeadi* and *G. tuberculifemur* in a 'simple experimental system' for 15 min showed that parasitism by *G. ashmeadi* was 12-54% higher than *G. tuberculifemur* for both egg ages (Figure 2).

Long exposure time

Approximately 50 GWSS eggs (one-two days of age) were placed in a double ventilated vial cage and exposed to one mated

female and male (24-48 h old) for either 24 h or five days. Approximately 20 replicates were set up for each exposure time. The number of male and female *G. ashmeadi* and *G. tuberculifemur* offspring were recorded for each vial. Figure 3 shows that parasitism by *G. ashmeadi* was 44-53% higher than *G. tuberculifemur* for both exposure times.

Results described in Figures 1-3 suggest that *G. ashmeadi* is superior to *G. tuberculifemur* under complex and simple experimental conditions with short and long exposure times. Results from competition experiments where both parasitoids are presented simultaneously to host eggs demonstrated that *G. ashmeadi* should outcompete *G. tuberculifemur* in the field, thereby possibly preventing widespread establishment and proliferation, of *G. tuberculifemur* in California. This result is similar to Irvin & Hoddle (2005b) who showed that *G. ashmeadi* was superior to *G. triguttatus* and *G. fasciatus* in laboratory studies investigating egg age preference, GWSS parasitism rates and adult parasitoid longevity. *Neither G. triguttatus nor G. fasciatus have performed well following mass releases in California where G. ashmeadi is present, which suggests that the results of these competitive lab experiments may accurately predict field performance. Despite substantial effort and significant cost being dedicated for several years to the mass rearing and distribution of these two poorly performing parasitoids.*

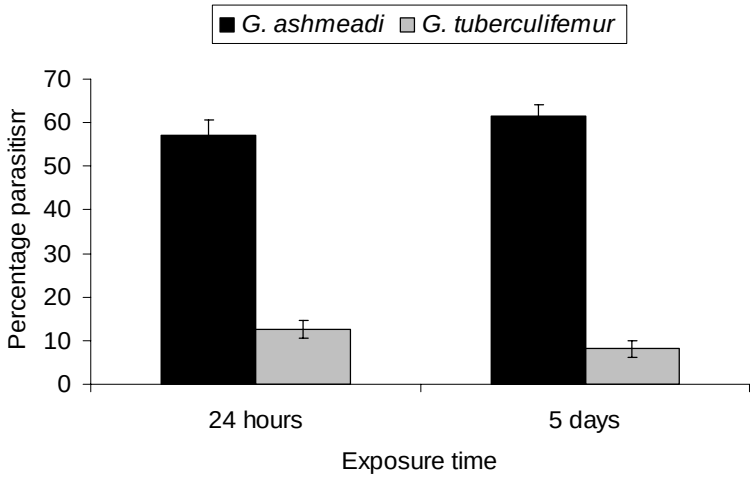

Figure 3. The mean percentage of *G. ashmeadi* and *G. tuberculifemur* offspring emerging when 50 GWSS eggs were exposed simultaneously to one mated female *G. ashmeadi* and *G. tuberculifemur* for 24 h *or* 5 days.

There was no significant difference in overall parasitism of GWSS eggs between vials containing *G. ashmeadi* only (A) and vials containing both *G. ashmeadi* and *G. tuberculifemur* (AT) for the complex system (Figure 4). This may be attributable to host availability since one female *G. ashmeadi* already reached maximum percentage parasitism (86-90%) and a small proportion of GWSS eggs are always lost through desiccation and superparasitism. For the simple system, parasitism remained at 55-60% regardless of whether vials contained one *G. ashmeadi* (A), two *G. ashmeadi* (AA), or one *G. ashmeadi* and *G. tuberculifemur* (AT) (Figure 5). This may be attributed to conspecific presence increasing non-ovipositional behavior such aggressive chasing and antennating conspecifics, thereby reducing time available for oviposition.

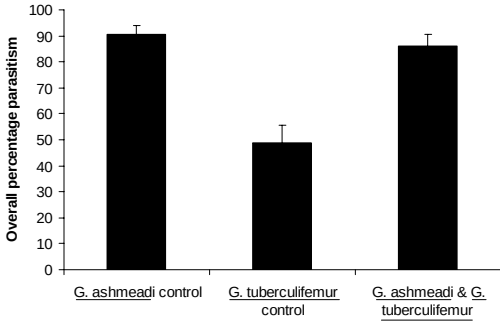

Figure 4. Overall percentage parasitism of GWSS egg masses exposed to three treatments in a 'complex experimental system' for 1 h.

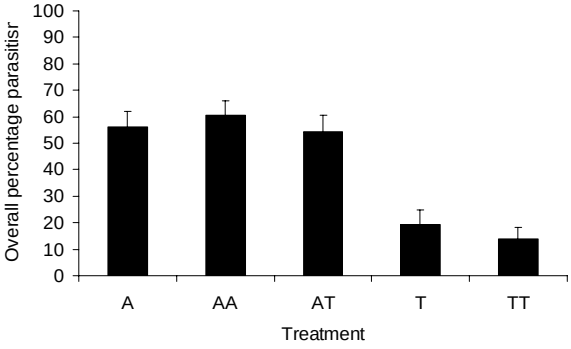

Figure 5. Overall percentage parasitism of GWSS egg masses exposed to five treatments in a 'simple experimental system' for 15 min (see Figure 2 for treatment legend).

Table 1 shows that female *G. ashmeadi* and *G. tuberculifemur* allocated up to 1.3% of time to each aggressive behavior when they were presented with a conspecific. *These results may suggest that the introduction of G. tuberculifemur in California may not benefit biological control of GWSS because no additive or synergistic effect on parasitism of GWSS eggs is observed in the laboratory.* Alternatively, the parasitism rates observed in treatments A, AA and AT (55-60%) may indicate that 60% is the maximum mean parasitism obtainable in this experimental design since GWSS eggs were exposed for 15 min, and there were always a small proportion of replicates that resulted in no parasitism which reduced overall parasitism estimates.

Table 1: The percentage of time allocated to eleven behaviors when *G. ashmeadi* and *G. tuberculifemur* were exposed to one GWSS egg mass for 15 min in five experimental treatments (see Section 3.1.2 and Figure 2 for behavior and treatment legends).

Behavior	Treatment					
			AT			
	A	AA	A	T	T	TT
SC	18.96 ± 4.26	21.14 ± 2.93	19.13 ± 3.36	38.92 ± 6.49	42.88 ± 6.22	39.44 ± 4.22
SL	8.12 ± 2.17	18.02 ± 2.22	16.12 ± 3.57	18.06 ± 4.30	8.00 ± 1.98	18.00 ± 2.69
SE	11.04 ± 1.73	8.43 ± 1.23	9.89 ± 1.63	7.74 ± 1.54	6.66 ± 1.91	6.77 ± 1.22
O	42.08 ± 5.56	32.5 ± 2.58	37.41 ± 4.61	22.79 ± 4.84	29.11 ± 6.17	14.00 ± 2.84
R	10.83 ± 4.22	10.1 ± 2.67	8.81 ± 3.21	5.59 ± 2.56	7.55 ± 3.07	11.00 ± 2.87
G	8.33 ± 3.16	4.37 ± 1.18	4.08 ± 1.89	3.87 ± 1.41	5.77 ± 2.01	8.33 ± 1.54
C	0 ± 0	0.10 ± 0.10	1.29 ± 0.65	0.21 ± 0.21	0 ± 0	0.33 ± 0.33
AC	0 ± 0	1.14 ± 0.38	0.64 ± 0.35	1.29 ± 0.48	0 ± 0	0.88 ± 0.29
SETS	0 ± 0	1.14 ± 0.68	0.86 ± 0.51	0.43 ± 0.29	0 ± 0	0.22 ± 0.15
OTS	0 ± 0	2.29 ± 1.43	1.29 ± 1.09	0.86 ± 0.67	0 ± 0	0 ± 0
F	0.62 ± 0.45	0.73 ± 0.36	0.43 ± 0.29	0.21 ± 0.22	0 ± 0	1.00 ± 0.52

Functional response

The functional response (Type I, II, or III) is a measure of how many hosts a parasitoid can process in a given exposure time. Handling time and area of search are the two most significant quantifiable factors that affect the shape of the functional response curve. Figure 6 shows that both *Gonatocerus* species demonstrate a type II functional response curve (number of hosts attacked per unit time decreases as host density increases), and that *G. ashmeadi* outperformed *G. tuberculifemur* at all host densities greater than five eggs.

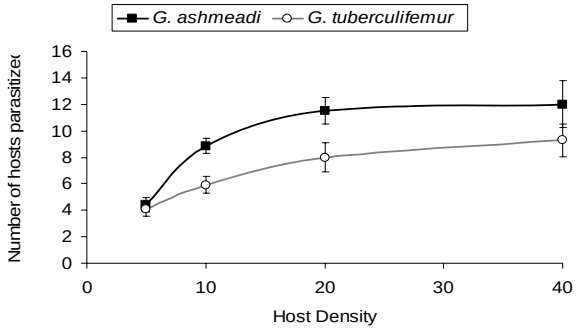

Figure 6. The relationship between host density and the number of GWSS eggs parasitized by a single female *G. ashmeadi* or *G. tuberculifemur* during a one hour exposure period at 26°C.

CONCLUSIONS

Preliminary results of studies completed thus far suggest that the potential impact of releasing *G. tuberculifemur* in California on the biological control of GWSS may not out-weigh the cost of mass rearing and releasing *G. tuberculifemur* in California. When time and labor costs for large-scale colony maintenance, long-term concerns about non-target impacts in California, disruption of existing levels of control, and potential invasion by *G. tuberculifemur* back into the southeast USA where GWSS originated are all considered there appears to be no quantifiable benefit to releasing *G. tuberculifemur* in California for the biological control of GWSS.

The experiments addressing the five objectives outlined in this report will provide important biological data on the neoclassical biological control agent, *G. tuberculifemur*, while in this parasitoid is still in quarantine. Work presented in this report demonstrates that *G. tuberculifemur* may be inferior to *G. ashmeadi*, and this would suggest no advantage to releasing this neoclassical agent from quarantine. A decision not to release based on these assessments would negate potential long-term concerns about non-target impacts in California (i.e., against *H. liturata*, the smoketree sharpshooter), possible infiltration of the home range of GWSS, and interference and reduction of current levels of biological control achieved with the resident natural enemy guild of old association parasitoids.

REFERENCES

Irvin, N. A., Hoddle, M. S. (2005a). Determination of *Homalodisca coagulata* (Hemiptera: Cicadellidae) egg ages suitable for oviposition by *Gonatocerus ashmeadi*, *Gonatocerus triguttatus*, and *Gonatocerus fasciatus* (Hymenoptera: Mymaridae). Biological Control 32: 391-400.

Irvin, N. A., Hoddle, M. S. (2005b). The competitive ability of three mymarid egg parasitoids (*Gonatocerus* spp.) for glassy-winged sharpshooter (*Homalodisca coagulata*) eggs. Biological Control 34: 204-214.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.