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

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ABSTRACT
To date, we have conducted experiments investigating the egg age preference and competitive ability of clade 1 (Hoddle and Irvin 2007) and clade 2 (presented here), *G. tuberculifemur* against *G. ashmeadi*. Results strongly suggest that *G. ashmeadi* is superior to *G. tuberculifemur* clades 1 and 2 when parasitizing GWSS eggs in "complex" and "simple" experimental conditions with short and long exposure times. The higher rates of parasitism and larger host age range demonstrated by *G. ashmeadi* suggest that this species will be more competitive than *G. tuberculifemur* clades 1 and 2 in the field when competing for GWSS egg masses and will likely prevent the widespread establishment of *G. tuberculifemur* clades 1 and 2. Consequently, it would appear that *G. tuberculifemur* (clades 1 and 2) will contribute little to the biological control of GWSS in California.

INTRODUCTION
Gonatocerus tuberculifemur is a sharpshooter parasitoid from Argentina that attacks glassy-winged sharpshooter (GWSS) egg masses. This parasitoid is being considered for use as a neo-classical or new association biological control agent for GWSS in California. A recent molecular study (de León et al. 2008) has identified two distinct clades within the species currently considered to be *G. tuberculifemur*. Populations of *G. tuberculifemur* collected from San Rafael in Argentina cluster into clade 2 and the rest of the populations for which DNA was analyzed clustered into clade 1. There is substantial uncertainty about the safety of releasing these agents (i.e., unintended spread and non-target impacts) and whether these neoclassical natural enemies 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. The purpose of this work has been to ascertain in quarantine whether these neoclassical biological control agents from Argentina in clades 1 and 2 can outperform the dominant GWSS parasitoid in California, *G. ashmeadi*. To date, we have completed experiments assessing clade 1 and a summary of these results were presented in the 2007 Pierce's Disease Symposium Proceedings. This report presents experimental results for "*G. tuberculifemur*" that are representative of populations that compose clade 2.

OBJECTIVES
1. Ascertain oviposition preferences of *G. ashmeadi* and *G. tuberculifemur* clade 2 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.

RESULTS
Objectives 1 & 2: Egg age preferences and competitive ability

Experiment 1 - Complex environmental system:
One mated female *G. ashmeadi* and *G. tuberculifemur* (~24-36 h of age) was presented simultaneously with one GWSS egg mass (composed of ~4-8 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°C for emergence of parasitoids and GWSS nymphs. The number of emerged and unemerged males and females of each parasitoid species was 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* competing for GWSS eggs. Results from vials containing one egg mass exposed simultaneously to one *G. ashmeadi* and *G. tuberculifemur* showed that parasitism by *G. ashmeadi* was consistently 53-81% higher than *G. tuberculifemur* for all three egg ages (one, three and five days of age) tested (**Figure 1**). Results from the control vials (those containing only one female) showed that *G. tuberculifemur* demonstrated no egg age preference, parasitizing 7% of eggs one, three and five days of age (**Figure 1**). Results for the *G. ashmeadi* controls showed that GWSS egg parasitism ranged from 58-82% (**Figure 1**). Statistical analyses are currently underway to determine whether egg age

had a significant effect on parasitism by *G. ashmeadi*. The higher rates of parasitism demonstrated by *G. ashmeadi* suggest that this species will be more competitive than *G. tuberculifemur* in the field when attacking GWSS egg masses.

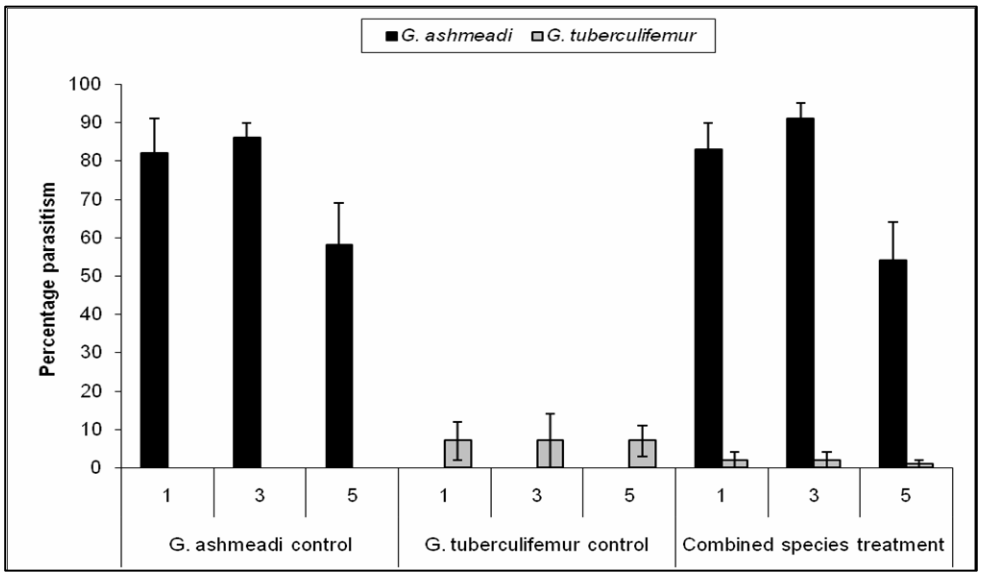

Figure 1. Percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* resulting when GWSS egg masses aged one, three, and five days of age were exposed to parasitoids either alone or in competition with each other.

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 2**). Female *G. ashmeadi* dominated the mixed parasitoid system and the contributions to overall parasitism by *G. tuberculifemur* were negligible.

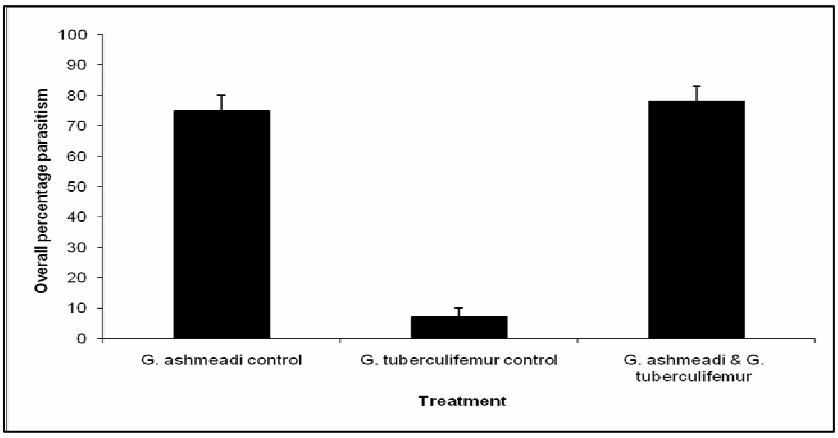

Figure 2. Overall percentage parasitism of GWSS egg masses exposed to three treatments in a 'complex experimental system' for 1 h.

Experiment 2 - Simple environmental system:
One mated female *G. ashmeadi* and *G. tuberculifemur* (~24-36 h) was presented simultaneously to one GWSS egg mass (~4-8 eggs, 1-2 days of age) on a single leaf in a double ventilated vial. This 'simple system' was replicated 15 times. Egg masses were not camouflaged amongst four other similar sized leaves. Exposure time of GWSS eggs to parasitoids was 15 minutes 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), and feeding on honey (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 to account for interaction effects independent of species.
Results from vials containing one egg mass exposed simultaneously to one *G. ashmeadi* and *G. tuberculifemur* in a 'simple experimental system' with just one leaf for 15 minutes showed that parasitism by *G. ashmeadi* was 61% (**Figure 3**), while *G.*

tuberculifemur parasitized no GWSS eggs within the 15 minute exposure time (**Figure 3**). Behavioral data are currently being analyzed.

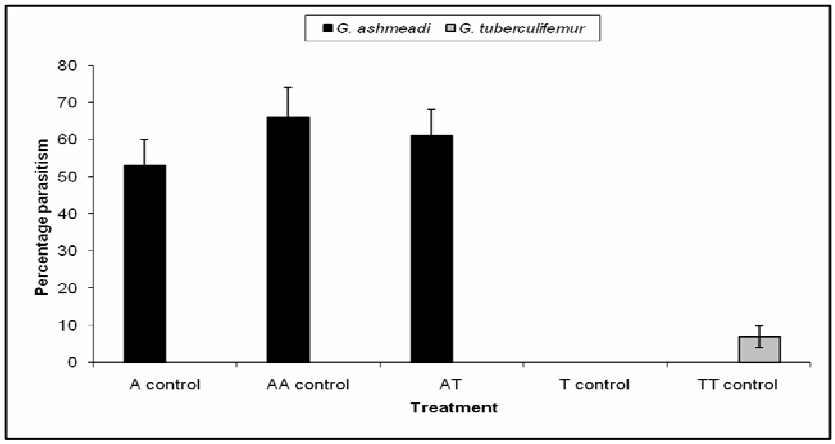

Figure 3. Percentage parasitism by *G. ashmeadi* and *G. tuberculifemur* resulting when GWSS egg masses aged 1-2 days of age were exposed to parasitoids either alone or with intraspecific or interspecific competition (A = vial containing one female *G. ashmeadi*; AA = vial containing two female *G. ashmeadi*; AT = one female *G. ashmeadi* and *G. tuberculifemur*, T = one female *G. tuberculifemur;* TT = two female *G. tuberculifemur*).

<u>*Experiment 3 - Long exposure time*</u>*:* When ~50 GWSS eggs were exposed to one female *G. ashmeadi* and *G. tuberculifemur* for either 24 hours or five days, parasitism by *G. ashmeadi* was 82-94% higher than *G. tuberculifemur* for both exposure times (**Figure 4**).

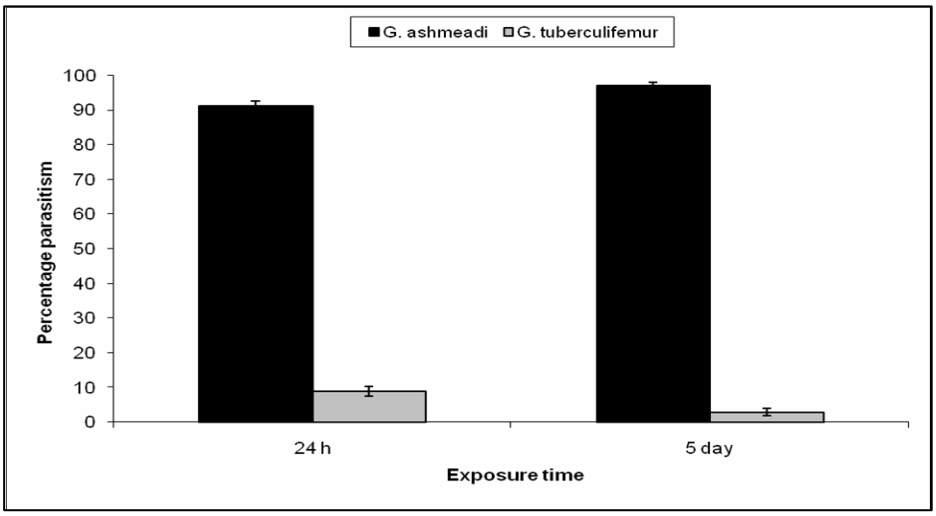

Figure 4. The mean percentage of *G. ashmeadi* and *G. tuberculifemur* offspring emerging when 50 GWSS eggs were exposed simultaneously to one mated female *G. ashmeadi* and *G. tuberculifemur* for 24 h or five days.

CONCLUSIONS

Gonatocerus tuberculifemur is a sharpshooter parasitoid from Argentina that is being considered for release from quarantine for biological control of GWSS in California. 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 (i.e., the southeast USA and northeast Mexico). By studying the egg age preference, competitive ability and functional response of *G. ashmeadi* and *G. tuberculifemur* (clades 1 and 2) we sought to determine which of these three parasitoids is likely to be the

most efficacious.

Results from experiments involving clade 2 of *G. tuberculifemur*, as shown here, suggest that *G. ashmeadi* is superior to *G. tuberculifemur* when parasitizing GWSS eggs in "complex" and "simple" experimental conditions with short and long exposure times. Results from competition experiments where both parasitoids were presented simultaneously to host eggs demonstrated that *G. ashmeadi* outcompeted *G. tuberculifemur* clade 1. These results suggest that *G. ashmeadi* may prevent widespread establishment and proliferation of *G. tuberculifemur* clade 1 in California. This result is similar to Hoddle & Irvin (2007) who showed that *G. ashmeadi* was superior to *G. tuberculifemur* clade 2, and to *G. triguttatus* and *G. fasciatus* under similar experimental conditions (Irvin and Hoddle 2005). Neither *G. triguttatus* or *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.

Our data thus far suggests that the potential impact of releasing *G. tuberculifemur* clade 2 (and clade 1) in California on the biological control of GWSS may not out-weigh the cost of mass rearing and releasing of this biological control agent. When time and labor costs for large-scale colony maintenance, disruption of existing levels of control achieved with the resident natural enemy guild (especially *G. ashmeadi*), 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* clade 2 in California for the biological control of GWSS. Similar conclusions have been reached from completed work on G. tuberculifemur clade 1 (Hoddle and Irvin 2007).

REFERENCES CITED

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