LREC Experiment – Navels
The ground around 45 individual trees of block 23 at the Lindcove Field Station were treated by backpack sprayer with the equivalent of 32 oz of Admire per acre on 30 Apr 2002 and 20 Jun 2003. An equal number of trees were left untreated. Before treatment, the 90 trees were chosen based on similar yields (1 March 2002). After the first treatment (11 February 2003), fruit were harvested and evaluated for number, size and quality. There were no significant differences in any of the parameters tested for the treated and untreated trees.

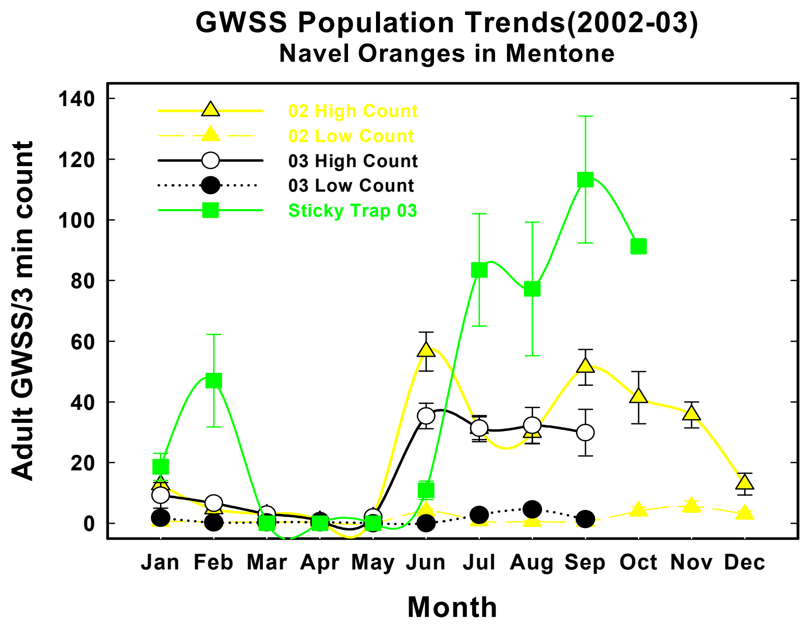

Figure 1. Means are based on nondestructive 3 min counts from 6 trees/replication/week. Each replication had 1 Seabright yellow panel trap. N = 5 ± SEM.

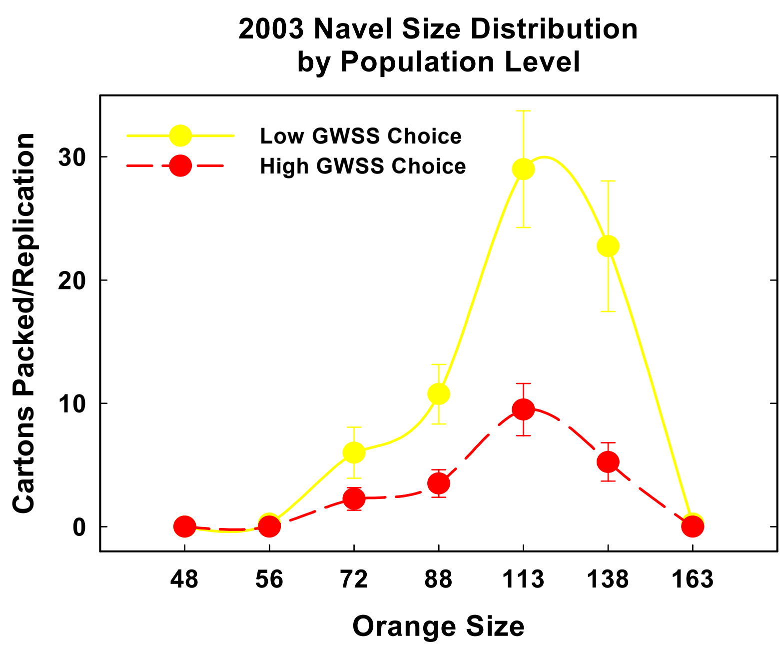

Figure 2. Mean numbers of export and choice cartons packed fresh between the high and low GWSS populations for the Jan 2003 Navels. Low population (virtually 0 GWSS) trees were treated on 7 April 2002. 5 reps (Each rep = 37 trees) ± SEM.

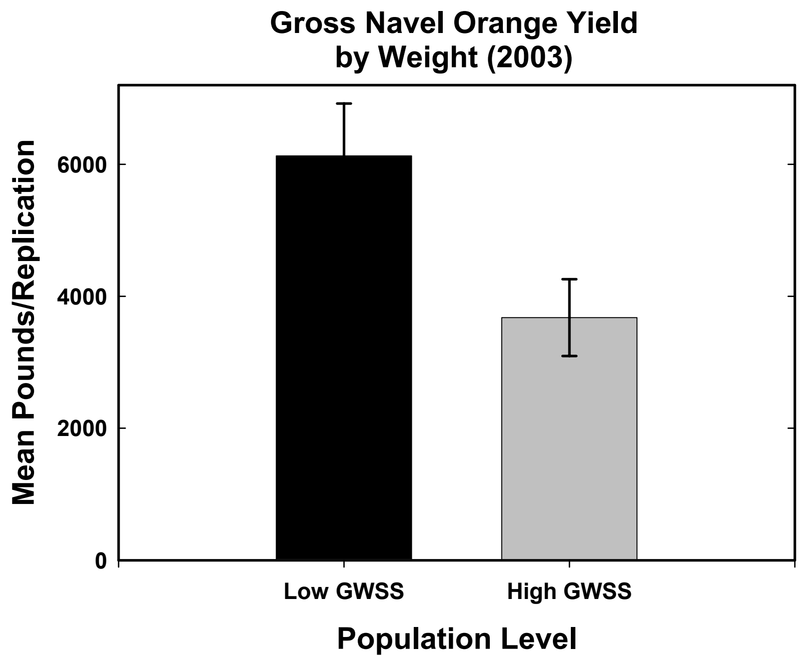

Figure 3. The low population trees produced more gross weight of navels than the high population trees. N = 5 ± SEM. 1 replication = 37 trees.

FUNDING AGENCIES
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COLD STORAGE OF PARASITIZED AND UNPARASITIZED EGGS OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA*

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Reporting Period: The results reported here are from work conducted from November 1, 2002 to October 1, 2003.

ABSTRACT
Excised leaves and cuttings of the *Euonymus japonica* plant were tested for cold tolerance over time at 2, 5, and 10° C in the dark for purposes of developing a protocol that would be favorable to cold storage of parasitized and unparasitized GWSS egg masses oviposited on the leaves of this plant. Cuttings generally survived longer than individual leaves at all temperatures tested, some as long as 165 days. The two lower temperatures were best for storage of the cuttings while 5°C was best for the individual leaves. Successful parasitism of GWSS eggs stored on euonymus cuttings for 20days at 13°C for both *Gonatocerus ashmeadi* and *G. triguttatus* was similar to control levels while parasitism steadily declined with longer storage periods. The threshold temperature for development of GWSS embryos appears to be approximately 12°C and, depending upon the age of the embryos when placed at 13°C, hatching can be as high as 36% after 40 days storage. We previously observed that *G. ashmeadi* within GWSS eggs stored for 7 days at 2°C were able to survive, however, our recent tests showed that neither *Gonatocerus* species were able to survive 20 days at this temperature.

INTRODUCTION
The glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* (Say), has become a serious economic problem in California as a vector of the xylem-inhabiting bacterium, *Xylella fastidosa* Wells (Sorensen and Gill 1996). Classical biological control typically relies on techniques of mass production of natural enemies to amass large quantities of individuals for eventual release and establishment of field populations (Miller 1995). Parasitoids in the families Mymaridae and Trichogramattidae are the most common natural enemies of GWSS in its native range (Turner and Pollard 1959) and in California (Phillips 1998). Three egg parasitoids, *Gonatocerus ashmeadi* Griault, *G. triguttatus* Girault, and *G. morrilli* Howard, are among the more important species being studied as agents for control of GWSS. Cold storage of insects during the rearing process of insects has proved to be a valuable aid to bio-control when implementing an IPM program. Low temperature storage of immature parasites within host eggs and storage of host eggs for later use have been extensively investigated (Leopold 1998). During the process of rearing and maintaining the GWSS and parasitoid colonies, it would be desirable to be able to store the GWSS egg masses and parasitized eggs for use at a later date.

In the absence of an effective artificial diet and ovipositional rearing system for *H. coagulata*, current rearing methods all require a constant supply of living plant materials. The GWSS females mostly deposit eggs into the lower epidermis of host plant leaves (Blua et al. 1999). Thus, the physiological status of these egg-bearing leaves has a direct impact on cold storage of GWSS eggs. Some host plants preferred for oviposition by the GWSS do not survive when placed under low temperature non-freezing conditions and subsequently, the developing insects also die (Leopold et al. 2002). While some of the intact host plant species survive cold storage relatively well, the space required for storing whole plants, each bearing a few egg masses, would be impractical when rearing large numbers of insects. Thus, many of the parameters that ensure vigor of the host plant leaves or cuttings during cold storage are yet to be defined.

Previous studies on short-term cold storage of GWSS eggs showed that the GWSS eggs could hatch in part or all after storage at 10º C for 1-6 days, but no eggs hatched after cold storage for more then 8 days at 10º C (Leopold et al. 2002). Another preliminary experiment conducted by Andress at al. (2000) indicated 67%, 16 % and 1% survived after 1, 2, 3, days respectively at 1ºC. Also, it has been suggested that GWSS eggs collected from different seasons may have different cold tolerance capabilities (Andress at al. 2000). However, data regarding seasonal changes and the effect of long-term cold storage on the development of the GWSS still remain unknown.

Although cold storage has been studied in many insects, there is still much to be learned about the effects of low temperatures on insect parasitoids (River et al. 2000). Two parasitoid wasps, *G. ashmeadi* and *G. triguttatus*, oviposit into GWSS eggs and their wasp progeny emerge within 15-20 days at 22º C. In the absence of practical techniques for propagation the wasps via artificial methods, the parasitoid wasps are mainly dependent on the provision of GWSS eggs collected in laboratory.

Apart from the internal factors controlling development of the parasitoids, other conditions including the developmental stage or age of the host, storage temperature, and humidity and lighting regimes may affect their development and/or survival. Preliminary experiments were conducted at 2 and 5ºC in fully dark environment in 2002 using a relatively low number of parasitized GWSS eggs (Leopold et al. 2002). This report presents current progress on investigations determining the cold tolerance of an ovipositional host plant and of parasitized and unparasitized eggs.

OBJECTIVES

1. Determine the effect of low temperatures on excised leaves and plant cuttings of host plants preferred by the GWSS for oviposition.
2. Determine the cold tolerance of GWSS eggs over time and parasitism by wasps on eggs that have experienced storage.
3. Determine effect of cold storage and age of GWSS egg on the emergence rate of egg parasitoids.

RESULTS AND CONCLUSIONS

Objective 1. Effect of low temperatures on excised leaves and cuttings of host plants

Excised leaves and cuttings of euonymus plants (*Euonymus* spp.) were examined for cold tolerance in constant temperature incubators set at 2, 5 and 10º C in the absence of light. Leaves were placed into slits cut into a water-soaked sponge, while all cuttings were placed vertically into plastic containers filled with water. About 50% of the excised leaves stored at 5º C survived for over 90 days, significantly longer than those stored at 2 or 10º C (Table 1). After 2 months, >42% of the leaves appeared chlorotic when stored at 2 ºC, 25% at 5º C and 75% at 10º C. Further, many excised leaves stored at 10º C died from the fungal or microbial infections. Euonymus cuttings generally lived longer than excised leaves. The LT_{50} for the cuttings ranged from 81 days stored at 10º C to nearly 100 days when stored at 5º C. Some even survived for more than 160 days. Cuttings stored at 10º C started to shed leaves after 7 weeks and were infected by bacteria or fungi after four weeks in storage. When stored at 13 and 14º C, the leaves of euonymus cuttings containing GWSS eggs were mostly covered by mildew after 30-35 days and were subsequently shed. Currently we are testing the effects of low level light administered to cold-stored cuttings and leaves using a short day photoperiod. We are also testing mildew retardants on cuttings placed in cold storage.

Table 1. Survival time of Euonymus leaves and cuttings exposed to low temperatures.

Temp.	Excised leaves				Cuttings			
	N	LT_{50} (days)*	Survival time (Mean ± SE)**	Range (days)	N	LT_{50} (days)*	Survival time (Mean ± SE)**	Range (days)
2 ºC	26	61.2	76.9 ± 3.0 a	64~112	20	84.5	96.3 ± 10.4 a	36~147
5 ºC	19	89.5	94.7 ± 6.1 b	64~134	15	99.1	104. 8 ± 9.9 a	40~165
10 ºC	16	60.0	74.4 ± 5.1 a	40~115	22	81.2	87.4 ± 4.4 b	48~110

* LT_{50} represented the storage time caused to kill 50% of the leaves or cuttings at a given temperature.
**Values followed by different letters in each column were significantly different ($P < 0.05$)

Objective 2. Cold tolerance of GWSS eggs over time and parasitism by wasps

To determine the effect of low temperature on the GWSS hatching and parasitism by the two parasitoids, GWSS egg masses deposited on euonymus cuttings were stored at 12, 13 or 14ºC for varying lengths of time at 1-2 days post oviposition. After being taken out of incubators, the egg masses were then exposed to either of the two wasps, *G. triguttatus* or *G. ashmeadi*, for 2 days at room temperature (22 ºC) and under a L 10: D 14 photoperiod. Before statistical analysis, all data were square-root transformed to correct non-normality because the number of eggs/mass size was not constant. Of the GWSS eggs stored at 13º C for 20, 30, 35 or 40 days, eggs stored for 20 days had approximately 85% successful parasitism (Successful parasitism is defined as the number of wasp offspring ×100 / the number of the GWSS eggs) (Figure 1). The parasitism of the eggs stored for 30 and 35 days was significantly lower than that of the control and those stored for 20 days. Only 23% GWSS eggs stored for 40 days were parasitized by *G. triguttatus*. The percentage parasitism was negatively correlated to the storage times of the GWSS eggs at 13º C (Figure 2). The wasp, *G. triguttatus*, successfully parasitized nearly 42% and 39% of GWSS eggs stored at 12 ºC for 30 and 40 days, respectively and only 2% for those eggs stored for 80 days (Figure 3). After storage at 14 ºC for 20 days, up to 89% GWSS eggs was parasitzed by *G. triguttatus*, significantly higher than those eggs for 40 days (29%) (Figure 4).