A. PROJECT TITLE: Assessing the Post-Winter Threat of Glassy-winged Sharpshooter Populations

B. CDFA CONTRACT NO.: 07-0177

C. REPORTING PERIOD: Results reported here are from work conducted from March 2008 through July 2008.

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E. OBJECTIVES AND ACTIVITIES CONDUCTED TO ACCOMPLISH OBJECTIVES

Objective 1: Verify impacts of winter temperatures on GWSS survival from selected California sites.

The data collection portion of this objective was conducted on the campus of the University of California at Riverside, where access to live GWSS would be easier and not a quarantine issue. Dr. Hannah Nadel oversaw the study. The study ran from November 2007 through April 2008. Nine temperature cabinets were used for the study. Cabinets (Percival Scientific, Inc., Perry, IA) were programmed to run various fluctuating, diurnal temperature patterns that are representative of historical patterns from selected sites within California. For nine CIMIS sites (e.g., McArthur, Merced, Porterville, Gerber, Oakville, Davis, Santa Ynez, Arvin, and Riverside), mean hourly temperatures were calculated for the months of November, December, January, February, and March. Ten field-collected GWSS adults (5 mated pairs) were caged and held under a given temperature regime (e.g., Merced) for a five month period. Cylindrical cages were made of clear 4 mil polyethylene bags (30 x 76 cm) (U.S. Plastic Corp., Lima, OH) with two nylon mesh windows (14 x 18 cm) for airflow. A 0.3 cm thick layer of white sand was sprinkled on the soil surface to facilitate the search for dead insects, after which the cages were placed over the plants and fastened tightly onto the pot rim with a band of hook-and-loop tape.

In chronological order (November, December, January, February, and March), the temperature cabinets were programmed to simulate the mean daily patterns for the individual months (i.e., 30 days for November, 31 days for December, 31 days for January, etc.). These daily temperature patterns were simulated as 24-step programs (hour-by-hour) unique to each cabinet for each winter month. Simulated minimum temperatures were kept at or above 3°C to avoid GWSS and plant mortality due to freezing. Average relative humidity patterns were also simulated. The photoperiod was constant across cabinets throughout the study, with light provided from 6 AM to 6 PM by four 40-w fluorescent tubes and two 15-w incandescent bulbs. Temperature and RH were recorded by a data logger (Onset Computer Corp., Bourne, MA) placed in each cabinet. Using these temperatures, we determined how long it takes the GWSS adults to die under these conditions. The daily cooling degree-days (CDD) were calculated using the mean maximum temperature (T_m) corresponding to monitoring period and using the following formula:

$$\text{Daily CDD} = | T_m - 10 |, \text{ if } T_m < 10^{\circ}\text{C} \qquad [1]$$

$$= 0, \text{ if } T_m \geq 10^{\circ}\text{C} \qquad [2]$$

where 10ºC is the threshold temperature below which the GWSS feeding was inhibited and $| T_m - 10 |$ = the absolute value of the difference between the mean daily temperature and the feeding threshold temperature.

Adult GWSS were collected by beat-netting from lemon trees at the UCR Agricultural Operations citrus orchard in Riverside, CA, between late October and early December 2007. They were held on potted sweet orange and prostrate acacia in mesh and vinyl cages (BioQuip, Rancho Dominguez, CA) in a greenhouse at 25 $\pm$ 4°C with natural light (supplemented with sodium vapor lamps L:D 12:12) for 4 – 7 days before use.

Two plant species were selected as winter hosts for the study, ‘Washington Navel’ orange (*Citrus sinsensis* [L.] Osbeck) grafted on trifoliate orange (*Poncirus trifoliata* [L.] Rafinesque) rootstock, and prostrate acacia (*Acacia redolens* Maslin cv ‘prostrata’). Grapevines were not used as originally planned because of difficulty locating nursery stock not treated with insecticides. Prostrate acacia is a leguminous evergreen shrub that is an overwintering host for GWSS (H. Nadel, personal observation). One orange (75 cm tall) (TreeSource Citrus Nursery, Exeter, CA) and one acacia (Parkview Nursery, Riverside, CA) were potted together in a 180 cm^2 (7-inch) pot and acclimated at least 1 month in a greenhouse before the study started. A 10-day study revealed that the nursery plants were apparently free of toxic residues.

Exposure of GWSS to simulated November temperatures began on the following dates: Riverside 11/9/07; Arvin 11/14/07; McArthur, Oakville and Merced 11/16/07; Porterville, Gerber and Davis 11/30/07; and Santa Ynez 12/6/07.

Five male and five female GWSS were transferred in vials from holding cages to each experimental cage. Seven cages were placed individually in water saucers in each temperature cabinet and the plants and insects allowed to acclimate at 18°C for 24 hours before winter temperature simulations began.

GWSS mortality was recorded weekly. Cages were removed from temperature cabinets only long enough to examine and remove dead insects, and were quickly returned (2-5 min). Insects that appeared to be dead were removed from cages, placed on paper under room temperature (20-21 °C), and covered with a clear vial. Those that did not revive within 2 hours were recorded as dead; revived GWSS were returned to their respective cages. Examination of cages was done during the warmest hours of the simulated day, when the insects were likely to show movement. Dead insects were counted and sexed. The potting medium was kept moist with weekly or biweekly watering, as needed.

Numbers of live and dead individuals were counted weekly until all insects died or the 5-month study period ended. Each temperature regime was been replicated 7 times. The cumulative CDD_{GWSS} were calculated for each location regime (e.g., Merced) based on temperatures recorded with HOBO recorders within the temperature cabinets. We are currently completing the statistical analysis of percent survival compared among regimes using a Repeated Measures ANOVA. The numbers of cumulative CDD_{GWSS} required to kill various percentages (e.g., 50%, 90%, 95%) of GWSS individuals per cage will be compared across location regimes to determine if the value to kill all test insects remains fairly constant across different diurnal temperature patterns.

Objective 2: Quantify and compare variation in "cooling degree day" accumulation within and among selected California sites using historical temperature data; and

Historical temperature data (last 10 years) was used to quantify and compare variation in "cooling degree day" accumulation within and among selected California sites. For 20 CIMIS sites, the monthly accumulation of CDD_{GWSS} will be calculated for the individual months of November, December, January, February, and March for each winter season examined (e.g., winters of 1996-1997 through 2006-2007). We have completed this exercise for the sites that we used as model temperature regimes in Obj. 1. We need to complete 13 more sites. We will statistically compare the sites and individual months to quantify the amount of variation in the accumulation of CDD_{GWSS} among sites and within sites. This exercise will provide insights into the amount of variation that occurs relative to probable survival of overwintering GWSS populations in various regions as a result of low temperatures. Based on our findings, it may be possible to reduce the number of regions in California that must be monitored for GWSS establishment. Certain northern or high altitude areas may consistently have temperatures so low that even annual temperature variation will not produce conditions under which significant numbers of GWSS individuals would survive the winter cold. Additionally, we should be able to compare our estimates of cumulative CDD_{GWSS} with historical CDFA records on GWSS sticky trap counts within specific areas. Dr. Mark Sisterson reports that CDFA will provide us access to their records on GWSS sticky trap counts within specific areas. We expect to find low numbers of GWSS trapped in those areas with high cumulative CDD_{GWSS} values.

Objective 3: Construct Geographical Information Systems (GIS) maps that estimate GWSS survival during the winter period.

Using temperature data collected between the months of November 2007 to March 2008 by CIMIS and the Western Regional Climate Center (WRCC), we will estimate the accumulation of CDD_{GWWS} for about 340 temperature monitoring sites. We will then construct GIS maps of

California that show: 1) the variation in cumulative CDD_{GWSS}; and 2) the estimated the risk of GWSS populations surviving the winter period. Spatial statistics techniques using ESRI ArcGIS® Geostatistical Analyst will be used to create interpolated surface maps using one of two analysis strategies: Inverse Distance Weighted or Krig surface generation. Risk will be expressed as a simple rating system such as: 0 = less than 0.1% possibility of the GWSS population surviving; 1 = possibility of between 0.1 and 1.0% of the GWSS population surviving; 2 = possibility of between 1.0 and 5.0% of the GWSS population surviving; and 4 = possibility of greater than 5% of the GWSS population surviving. For the time being, regions with ratings greater than 0 would probably require allocation of resources for GWSS suppression. However, standard GWSS monitoring should continue in all areas where GWSS populations are routinely found or might be expected to appear (e.g., areas along a transportation corridor, e.g., Hwy 65 in Tulare County). With an improved understanding of the climatological limits of GWSS overwintering survivorship, these risk estimates can help to spatially define where GWSS can be expected to persist in the agricultural landscape and identify where continued management efforts can be directed to limit introductions into currently non-infested areas.

To obtain an idea of what a GWSS survival map would look like, we used temperature data from 2006-2007 for the months of November and December 2006 and January thru March 2007. Using the techniques described above, a map was generated that showed GWSS mortality.

F. RESEARCH ACCOMPLISHMENTS AND RESULTS

Objective 1: Verify impacts of winter temperatures on GWSS survival from selected California sites.

The mean weekly temperatures sustained in the programmable environmental chambers generally dipped in the weeks (5 through 12) corresponding to December and January (Fig. 1). Higher-than-desired temperatures inadvertently occurred during weeks 2-4 in the Porterville chamber and in week 5 of the Merced chamber (Fig. 1). As planned, the mean temperature in the Riverside chamber was sustained at ≥ 10°C (i.e., above the lower feeding threshold of adult GWSS). In addition, the maximum (not shown) and mean temperatures in the McArthur chamber were successfully sustained below the feeding threshold. Survival was low or absent after simulated winter environments in all cases (Fig. 2, Table 1). As expected, all GWSS died rapidly (within five weeks) in the coldest cabinet that simulated McArthur conditions (Fig. 2), where the temperature never rose above the minimum feeding threshold (10°C). Presumably the insects in this chamber died of starvation, not freezing, because temperatures were maintained above 2.9°C. Nearly all GWSS adults in the McArthur chamber dropped to the soil beneath the plant canopy and appeared to have little opportunity to feed. The early 100% mortality in the Oakville and Davis simulations was unexpected, because the mean temperatures in those chambers were similar to the five other simulations that concurrently had substantial survival. However, Oakville temperatures were the third lowest of all in the first 4 weeks of the study, which may have hastened mortality. Davis temperatures were intermediate among several other simulations. Santa Ynez temperatures were somewhat lower than Oakville's during the first 4 weeks, and mortality was higher at that time (Fig. 2). Santa Ynez mortality was markedly more rapid than all, except McArthur during the first 8 weeks.

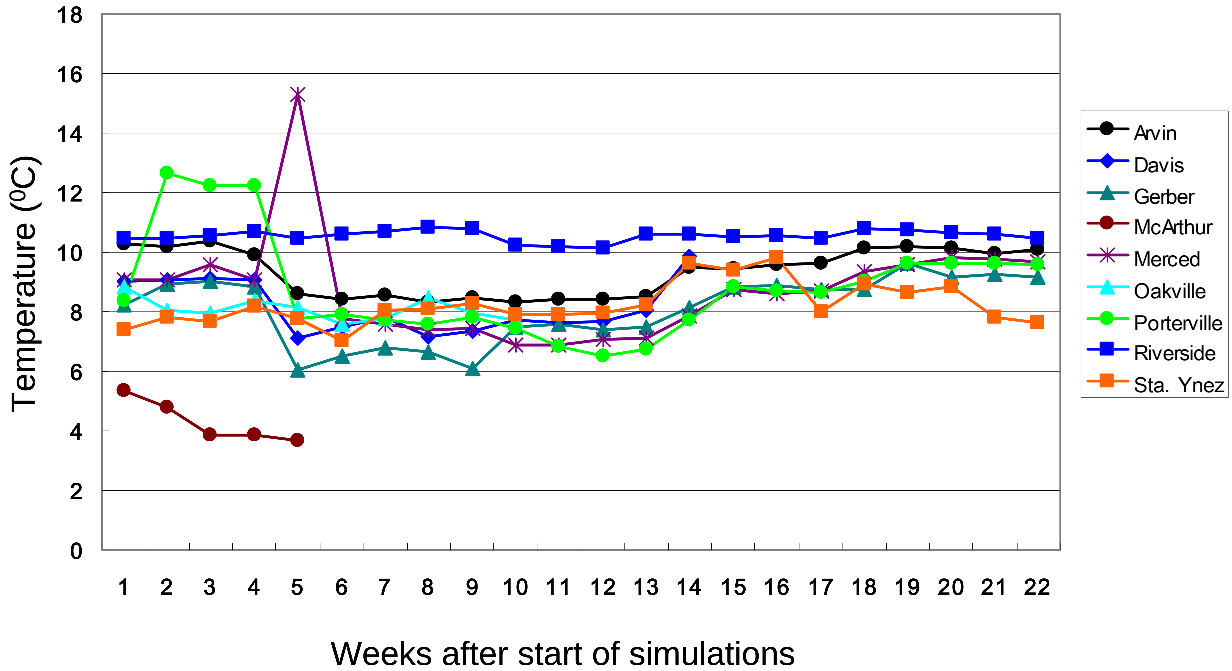

Fig. 1. Mean temperatures in each environmental chamber representing various CA sites (e.g., Arvin, Davis, etc.) throughout the study period.

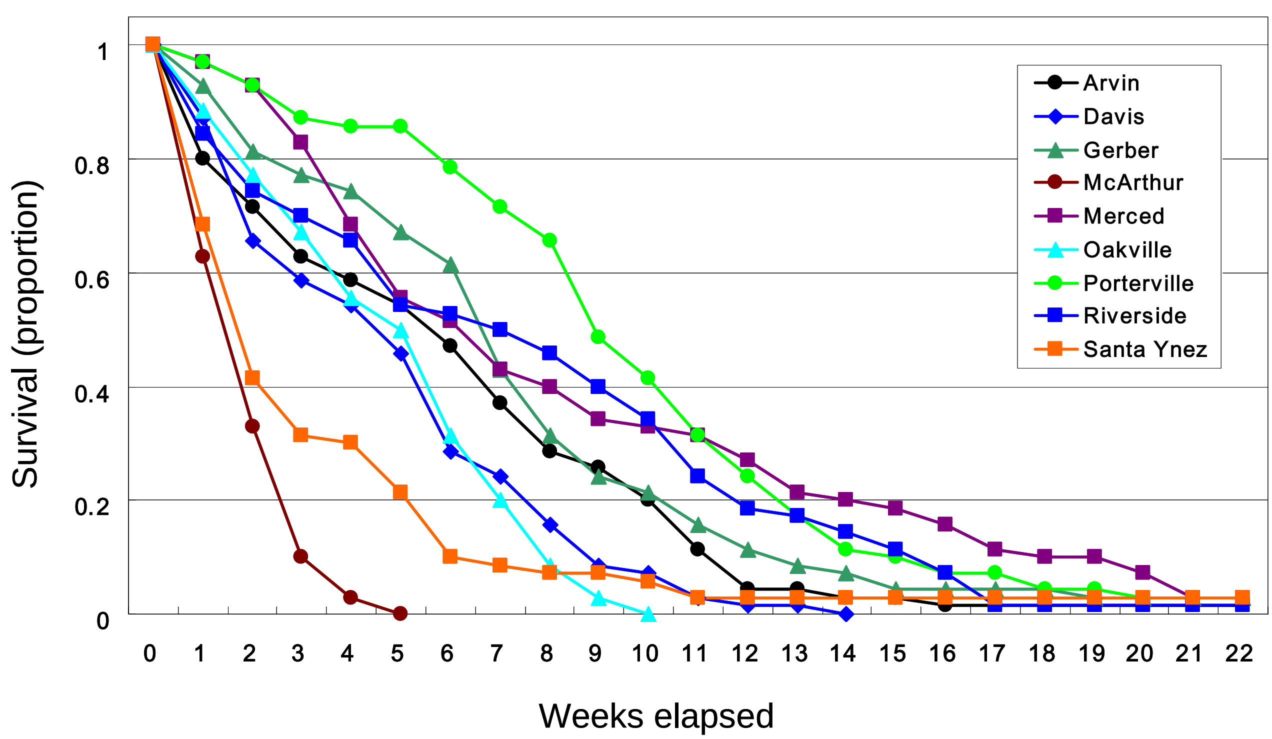

Fig. 2. Mean GWSS survival over the 22-week period (i.e., November through March) in each simulated site (e.g., Arvin, Davis, etc.).

Table 1. GWSS adult mortality and total CDD (based on a feeding threshold of 10°C) accrued to reach > 95% mortality.

CA Site	Accumulated CDD	Mortality (%)	Weeks elapsed
Riverside	0	98.6	17
Arvin	86	95.7	12
Oakville	117	97.1	9
McArthur	144	97.1	4
Davis	145	97.1	11
Santa Ynez	154	97.1	11
Porterville	221	95.7	18
Merced	236	97.1	21
Gerber	237	96.7	16

An attempt to predict GWSS overwintering mortality using a CDD model was advanced during prior work. Data were obtained from studies of caged GWSS in the field in Bakersfield and Riverside, and showed ~1% post-winter survival in those areas. The model used 10°C as a lower feeding threshold and explained 76% of observed variation. It predicted that 90 and 100% of GWSS mortality would occur at 143 and 215 CDD, respectively. The current work aimed to confirm and refine the prior model. Post-winter survival (0 - 2.9%) was similar to that found in the earlier work. The numbers of CDD accumulated over the duration of the current study in each winter simulation were widely varied in amounts and patterns (Fig. 3A). On a weekly basis, accumulated CDD in the environmental chambers was generally highest in December and January (Fig. 3B), corresponding to reduced temperatures in those months. The Riverside simulation served as a control, with nearly zero accrued CDD. Total accumulated CDD ranged from ~0 in Riverside to 284 in Santa Ynez by the end of five months (22 weeks) or when 100% mortality occurred (Table 1). Mortality in all regimes exceeded 97% when simulations ended, even in the warmest regime (Riverside). Preliminary examination of the data reveals wide variation in the relationship between CDD and GWSS mortality. For instance, 90% mortality was reached at accumulated CDD ranging from 0 (Riverside) to 235 (Merced) (Fig. 4). This indicates that the CDD model of winter survival may need some refinement to provide a more meaningful predictive tool over the range of winter climates found in California.

The data suggest that other factors may affect mortality. One potential factor is the amplitude of the daily thermocycle. The amplitude (difference between maximum and minimum daily temperatures) was highest in Santa Ynez (ca. 20°C), while most other simulations had amplitudes of about 10°C. This may explain why Santa Ynez had the second highest mortality during the first two months, reaching 90% within 6 weeks (Fig. 2). Another factor may be that short periods of intense cold may cause mortality levels that are similar to mortality caused by longer periods of less cold temperatures.

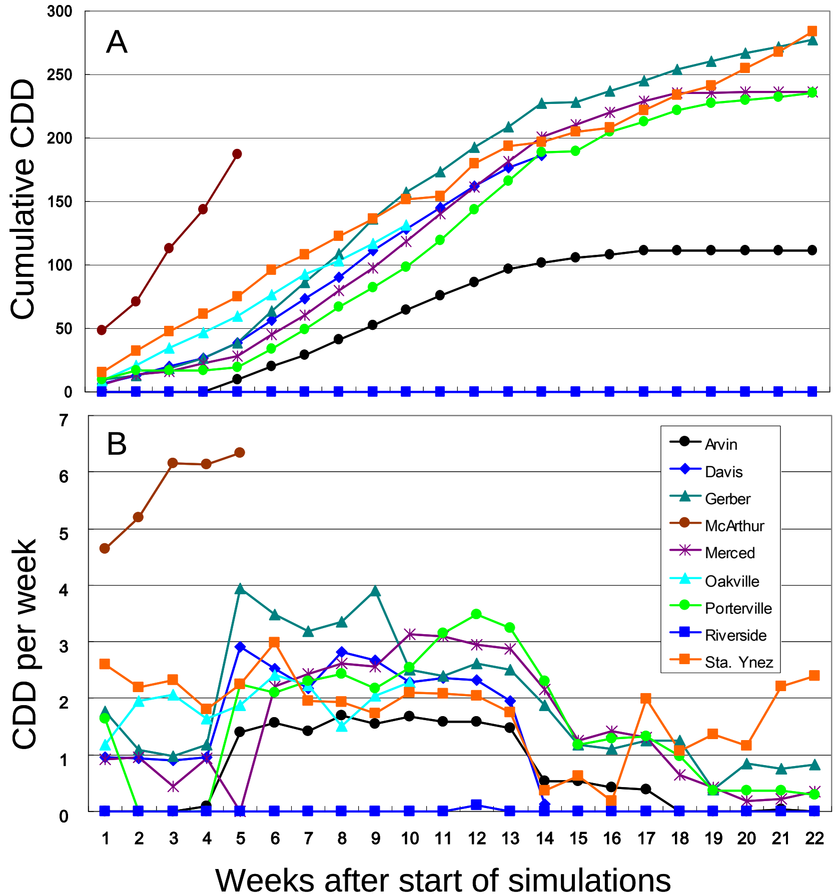

Fig. 3. Accumulated CDD (**A**) over the study period, and (**B**) CDD as they accumulate over the weeks, in each simulated environment.

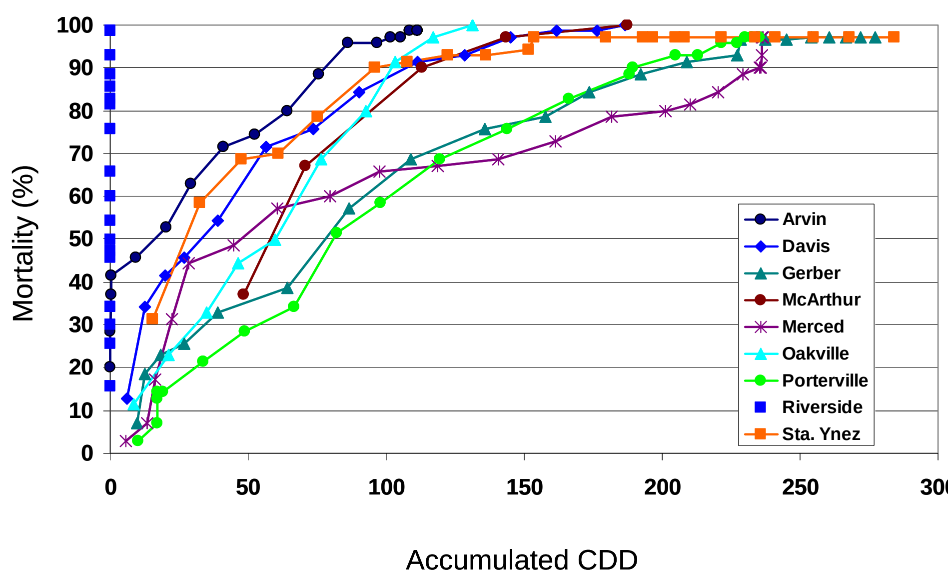

Fig. 4. Relationship between accumulated CDD and GWSS mortality under simulated winter conditions for nine California sites. Points represent mean weekly mortality data.

Examination of the data further suggests that adult GWSS mortality may be a function not only of temperature-mediated metabolic processes governing starvation, but also of temperature-mediated metabolic processes governing senescence. GWSS died throughout the study in the Riverside simulation (which did not fall below the 10°C feeding threshold but was near it) and during the first month in the Arvin simulation, although no CDD accrued during those periods (Fig. 2). The most likely explanation for this outcome is unhindered senescence of the GWSS, which were probably 3 to 4 months old at the start of the study in November 2007. Adult senescence and inability to feed, both mediated by temperature, probably interacted in a complex way to produce the observed variations in mortality patterns (Fig. 2). Because their metabolism slows with decreasing temperature, the aging of "cold-blooded" invertebrates is also generally slowed as temperature cools, prolonging life expectancy. On the other hand, starvation due to inadequate feeding in cool temperatures should hasten mortality. Both processes are complex, and if incorporated into the post-winter survival model, may help reduce unexplained variability. A post-winter mortality model incorporating starvation, senescence, and temperature will be developed if the data are adequate.

Objective 2: Quantify and compare variation in "cooling degree day" accumulation within and among selected California sites using historical temperature data.

Cooling degree days (CDD) have been estimated for the 9 sites used in our temperature cabinet simulations. The coldest site / month was McArthur in December with an accumulation of 513 CDD. The warmest site / month was Riverside in March with an accumulation of < 8 CDD. Within the San Joaquin Valley, Merced recorded the highest accumulation of CDD (185) in January. We will complete the proposed analysis of sites during the next research period.

Objective 3: Construct Geographical Information Systems (GIS) maps that estimate GWSS survival during the winter period.

Based on temperature data from 2006-2007, a map was generated that estimates various levels of adult GWSS mortality following the months of November 2006 to March 2007 (see Fig. 5). This map estimates that winter temperatures throughout much of California (designated in red) would kill less than 39% of the GWSS populations. One hundred percent mortality was estimated in the northeastern corner of the state (designated in dark blue). It must be remembered that our estimations only consider mortality resulting from the inability of the GWSS adults to feed because it is too cool for the feeding activity. Mortality due to the freezing and other factors (e.g., biological control, rain-induced drowning, wind, etc.) are not included. Additionally, mortality due to the natural aging (5 months) of the insects is not included. A new mortality factor was discovered during our studies: mortality that results when GWSS individuals fall from the host plant while it is cool and they are unable to climb back onto or locate their host plant. Insects may also be fed upon by predators such as birds while they lay on the ground.

For the next report period, a new map will be generated for the winter months of 2007-2008.

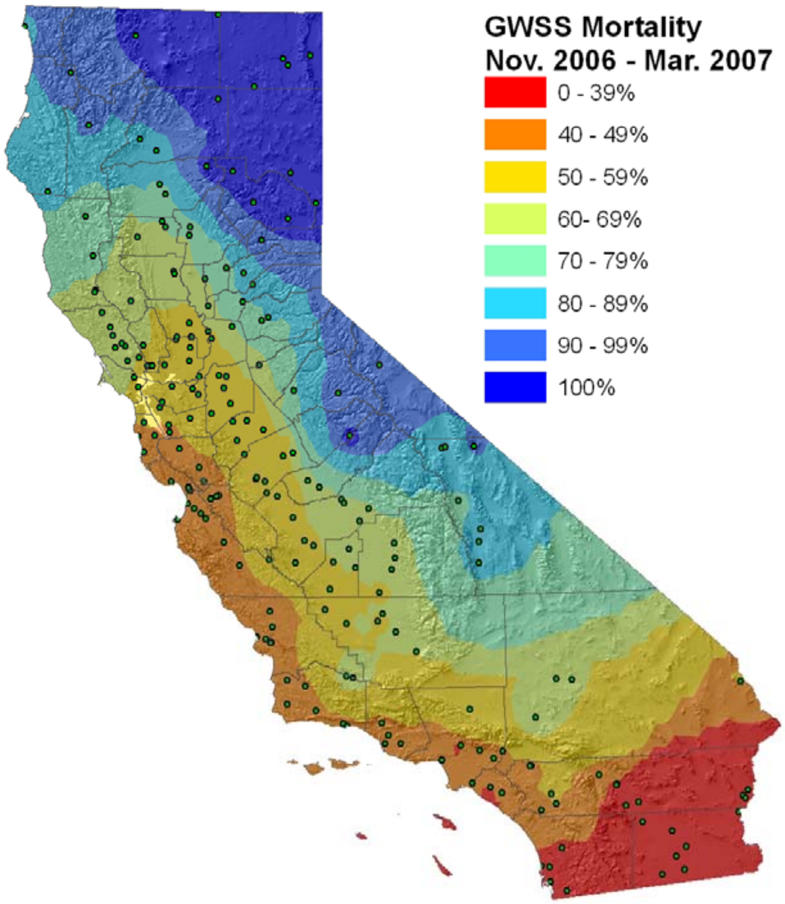

Fig. 5. Estimated percentage mortality of adult GWSS populations throughout California regions experiencing different levels of accumulated cooling day degrees from November 2006 thru March 2007. Dark blue represents 100% GWSS mortality and red represents 0 to 39% GWSS mortality. Green circles indicate CIMIS weather stations.

G. PUBLICATIONS, REPORTS, AND PRESENTATIONS

PUBLICATIONS

Son, Y., R. L. Groves, K. M. Daane, D. Morgan, and M. W. Johnson. Influences of temperature on *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) survival under various feeding conditions. Submitted to *Environmental Entomology* (Submitted May 2008), 37 pp.

REPORTS None.

PRESENTATIONS None.

H. RESEARCH RELEVANCE

Resources for GWSS management are limited and may dwindle as the threat of GWSS decreases with the advent of new management tactics and redirection of governmental and agricultural community resources to other exotic pests (e.g., Light brown apple moth, Asian citrus psyllid) that will appear as time passes. Findings from this project will potentially aid in the prediction of where GWSS populations will successfully overwinter within California and where they will not. With this knowledge, limited management resources can be better utilized in areas where a real GWSS problem exists and not wasted in areas where winter conditions have nullified threat of the pest for that year. However, because GWSS populations will expand and contract in size and geographical area each year and winter conditions vary annually, it will be necessary to monitor winter temperatures statewide each winter and then estimate the cumulative CDD to predict the GWSS threat to agriculture during the spring season.

I. SUMMARY OF SPECIFIC ACCOMPLISHMENTS

Prior research indicates that GWSS adults cannot feed at maximum daily temperatures below 50°F (= 10°C), thereby reducing its ability to survive cold winters. This study has verified the impact of cool temperatures on GWSS adults by exposing them to a regime of seasonal temperatures (within temperature cabinets) that reflect some of the marginal areas where we expect GWSS to poorly survive California winters. We have completed the laboratory experiments needed to verify the impact of cool temperatures on adult GWSS survival. Using temperature records to calculate numbers of cooling degree days, we plan to construct maps to delineate areas where post-winter GWSS mortality should be substantial, thereby providing a tool to estimate the springtime GWSS threat to different regions. We have provided an example of such a map (Fig. 5) in this report. If successful, post-winter GWSS survival maps could be produced each spring (e.g., April) that would provide estimates of where GWSS populations should be absent or minimal because of winter conditions.

J. SUMMARY OF INTELLECTUAL PROPERTY PRODUCED

To date, no intellectual property has been produced by the project.