KEYS TO MANAGEMENT OF GLASSY-WINGED SHARPSHOOTER: INTERACTIONS BETWEEN HOST PLANTS, MALNUTRITION AND NATURAL ENEMIES

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INTRODUCTION

Leafhopper vectors of *Xylella fastidiosa* (including GWSS) behave very differently from most herbivorous insects. GWSS and other xylophagous leafhoppers have evolved many unusual adaptations such as host switching to maximize nutrient uptake, unprecedented assimilation efficiency of nutrients, and excretion of ammonia that enable GWSS to subsist on xylem fluid. The physiology and behavior of GWSS that make it an efficient vector also make it less amenable to conventional management tactics. Adult GWSS may feed on hundreds of different host species, are long lived and exceptionally mobile and fecund. Natural seasonal fluctuations in plant xylem chemistry determine the seasonal use of host plants by GWSS adults. Xylem chemistry can be affected and/or manipulated by environmental factors, culture and management practices (fertilizer, water, pruning, rootstock) and weather extremes. Xylem fluid has the most dilute concentrations of dietary nitrogen and carbon of any plant tissue. Malnutrition is a primary source of mortality of immature GWSS. The nutrient requirements of immature GWSS are different and much more restricted than those of adults such that successful development might often require host switching. Whereas adult GWSS utilize many different host species, early instar nymphs survive and develop on only a few. GWSS females select both feeding hosts and ovipositional hosts, yet the interactions between these two choices may be complex and conflicting. We have established that adults prefer to feed on xylem fluid with specific chemical characteristics (high amide concentrations). However, nymphs develop poorly on these high amide diets. Nymphs develop more successfully on xylem fluid with low amide concentrations and proportionally higher concentrations of many of the more dilute amino acids that are deemed essential for the development of most insects. We have also established the physiological basis for this phenomenon: adults can efficiently use nitrogen and carbon from high amide concentrations, whereas young developing nymphs cannot. Females can consume more nutrients (thus, produce more eggs) on high amide diets, yet oviposition on these same hosts will result in up to 100% nymphal mortality (from malnutrition). Oviposition (as it affects parasitism rate) and survivorship of nymphs (malnutrition) are key mortality factors for GWSS with potential for manipulation to suppress leafhopper populations and *X. fastidiosa* diseases. It is these important key interactions and identification of the host plant species important in mediating such interactions that must be elucidated to manage GWSS in any geographical location.

OBJECTIVES

1. To determine the effects of host plant assemblages and host plant chemistry on distribution, performance and behavior of *Homalodisca coagulata*, glassy-winged sharpshooter (GWSS) and its natural enemies.
2. To determine the relationship of host plant xylem chemistry, and leaf morphology on host selection, feeding and ovipositional behavior of GWSS and its parasites.
   a. Assess host plant acceptance and subsequent feeding rate, host plant selection and acceptance for oviposition and the survival and performance of early and late instar nymphs as a function of host plant species.
   b. Quantify the impact of these plant variables on the behavior and parasitism rate of eggs by *Gonatocerus ashmeadi*.

RESULTS AND CONCLUSIONS

GWSS oviposits in many plant species, yet the majority of GWSS egg masses tend to be concentrated on a lower number of select host species that apparently offer the quality of xylem fluid (food) required for survival of nymphs. Food quality for nymphs appears to be an important factor affecting the population increase of GWSS. In this first year we concentrated on developing field methods and data towards determining the host selection behavior and use for oviposition of known host plants preferred for feeding by the adult GWSS.

Two experimental sites were established near Monticello, Florida. In north Florida, crape myrtle, *Lagerstroemia indica*, is an abundant and high quality host for GWSS adult feeding, but is clearly not a host species frequently used for oviposition. Therefore, one of the sites chosen was a uniform stand - ca. 10-15 acres - of crape myrtle as the adult feeding host. Four different species of host plants: holly (*Ilex* sp.), *Euonymus* sp., *Pyracantha* sp. and Bradford pear (*Pyrus* sp*.)* were chosen as potential oviposition hosts for the GWSS. Twenty trees of each species (a total of 80) were mixed and sorted into10 islands that were randomly distributed within the large block of crape myrtle to establish oviposition 'islands' for GWSS. Plants were fertilized and maintained under overhead irrigation that was supplemented by hand watering. Each plant on the islands and the surrounding crape myrtle were examined for the presence of GWSS adults and nymphs weekly. Every leaf on the plants was carefully examined every two weeks and GWSS egg masses found were counted, marked and dated, and left undisturbed. The experiment was established on 21 June and sampling continued until late September 2000. Yellow sticky traps were placed at each island to monitor flight and presence of GWSS adults near the islands.

The second site was also located at NFREC- Monticello. The experiment was established in a ca. 3-acre open turf-covered field surrounded on 3 sides by pecan and a forest on the other. Sampling began 14 June 2000 and was continued as described above until 28 September 2000. Ten different species of known GWSS host plants in pots and maintained as described above were grouped in 9 plant "islands". Each plant island contained several species of host plants in an effort to simulate the natural aggregated distribution of host plants as found in nature. A total of 149 trees were distributed among the 9 islands. All plants had drip irrigation. Because the islands were located in open grassland without other feeding hosts, crape myrtles were included in each island as food for GWSS adults. The plant islands were separated ca. 75m from each other. Yellow traps were placed as above to monitor GWSS adult presence and flight periodicity.

Results from both sites indicated a statistically significant preference of GWSS for laying eggs on holly plants over all other plant species offered as oviposition hosts. The second host most frequently used by GWSS for oviposition was Bradford pear. A few egg masses were also found on other hosts. *Pyracantha* was chosen as oviposition host only very early in the summer and in the fall. Parasites were able to utilize the GWSS eggs on all hosts.

To complement the small assemblages of mixed hosts blocks in Monticello-FL, a single large plot "epicenter" was constructed in Quincy, FL to test assemblages of hosts for oviposition and adult GWSS on a larger scale. A circular plot (500 m circumference) was constructed in an isolated grass-covered field in late June 2000. Eighteen ovipositional hosts (12 *Euonymus* sp. and 6 *Pyracantha* sp.) in containers were centered in the middle of the plot, and crape myrtle cv. Natchez in containers were arranged in concentric rings at 10, 20, 40, and 80 m away from the ovipositional hosts. The crape myrtles were arranged at 10 m intervals along the concentric rings. GWSS adults and nymphs were counted twice weekly from 1 July until leaves began to senesce (15 October). Remains of egg clutches were counted just prior to leaf senescence in mid-October on both the adult and ovipositional hosts. All plants were maintained by drip irrigation.

Common host species of GWSS were also established by in-ground planting in solid blocks in Quincy to provide data on seasonal fluctuations on a variety of host species. Host species established included peach, plum, apple, grape, holly, *Pyracantha* sp., citrus, *Euonymus* sp. and *Eucalyptus* sp. and also were maintained on drip irrigation. The grape planting included 21 cultivars of grapes (minimum of 6 reps per cultivar) to examine varietal selection within *Vitis* spp. Visual counts of all stages of GWSS were made one to two times weekly during the peak season (July through October) and once every one to two weeks after that to identify potential overwintering hosts.

Preliminary analysis of GWSS populations within the epicenter (circular grid) showed leafhopper aggregation, as is often noted for GWSS populations. However, aggregation patterns varied within the grid throughout the season and were not consistently related to distance from the center (ovipositional hosts). The most noticeable effect in the epicenter as compared to the contiguous blocks was that GWSS populations persisted on the potted crape myrtle well into the autumn. Populations in the solid blocks of crape myrtle declined by mid to late August, as we have previously documented on crape myrtle. In the epicenter, however, populations remained high (>50 % of peak populations) into October when crape myrtle leaves began to senesce. Counts of egg masses indicated that more egg masses were present on the 18 ovipositional hosts (*Pyracantha* and *Euonymus*) than on all 96 crape myrtle. Presumably, nymphs developing on ovipositional hosts may have supplied new adults in late summer that dispersed to the surrounding crape myrtle. Alternatively, the phenology and/or physiology of the potted plants was changed relative to the in-ground plants which maintained them attractive to GWSS for the extended time period. Exposure of these plants to GWSS was ca. 2 months longer than typically noted on crape myrtle. This suggests that proximity of adult and ovipositional hosts may greatly increase exposure of adult hosts to GWSS.

Counts of GWSS within solid blocks established that crape myrtle, apple and *Eucalyptus* were the predominant hosts throughout the summer. *Eucalyptus* was of particular interest as both nymphs and adults were present throughout the season. We note that in this initial year of planting, plant size of young plants may also have been a factor. Citrus seedlings, for instance, were not utilized as much as expected based on our previous examination of more mature citrus in our region. Similarly, grape was not used as frequently as expected by adult GWSS, but this may have (in part) reflected the size of first year plantings.

GWSS Rearing and Host Plant Parameters:
Our first-year efforts focused on standardizing and quantifying methodology for rearing GWSS. This methodology is essential for all research groups attempting to study GWSS development and nutrition, as well as for research on GWSS parasitoids. We have previously had good success in rearing GWSS, yet procedures need to be further standardized to allow year-round mass rearing of GWSS.

We have consistently achieved the highest success rates in rearing GWSS on glabrous *Glycine max*. This is not a naturally occurring host, as typically *G. max* has trichomes that inhibit feeding by GWSS (particularly by nymphs). Glabrous lines are, however, excellent and consistent rearing hosts. We have manipulated xylem nutrients by a variety of inoculation and fertilization techniques. Glabrous *G. max* grown under a variety of cultural conditions will support GWSS development. We have obtained best results if seeds are *Rhizobium* inoculated prior to planting, and only urea fertilizers are applied to soil. Recently we established that a variety of glabrous lines will support GWSS development, although there is some variation in developmental success with genotype. While some developmental success is noted for a variety of *G. max* genotypes and cultural conditions, combinations that result in comparatively low developmental rates often have a highly skewed sex ratio with a paucity of successfully developing females.

The biggest problem in rearing GWSS year round is strict attention to lighting conditions. In north Florida, GWSS that are field collected later than mid-September will diapause, whereas individuals collected in summer or reared under long day length conditions will continue to oviposit throughout the year as long as proper light conditions are maintained. When diapause occurs, we have recently established that we can break diapause if three weeks of short daylength are followed by long daylength (we employ 16:8 light:dark regime) *so long as* the proper ovipositional hosts are present.

Glabrous *G. max* can be grown in the greenhouse year-round, and is relatively pest free. Growth of *G. max* is slow under greenhouse/cage conditions and plants can be maintained for the length of a GWSS generation (ca. 35-50) days without having to replace within cages. While glabrous *G. max* is not a typical host of GWSS, they have provided excellent (and consistent) baseline data on developmental rates, instar durations, growth rates, consumption rates and other measurements on developing GWSS (we have also used these hosts to establish specific nutritional requirements of developing GWSS).

We initiated the second phase of this experiment, which is to assess these same insect measurements on California relevant host species. We have collected xylem chemistry data on the host plants used in the field plots and these are ready for analyses. We are currently investigating Chardonnay grapes, Navel oranges, Spanish Pink Lemon and Crape Myrtle. Data from these hosts will be compared to rates of development on *G. max* in order to assess the value of each of these species as developmental hosts for GWSS. Upon completion, other California relevant hosts will be examined with a similar protocol. These quantitative results are essential, as the California host list for GWSS continues to expand. Quantitative analysis is needed to prioritize the role of each potential host for implementation of GWSS control measures.

Natural Enemies:
By exposing GWSS eggs to parasite adults, we determined the duration of the susceptibility of GWSS eggs to the parasites *Gonatocerus ashmeadi and Gonatocerus morrilli.* It was determined that parasitoids can successfully parasitize 100% of GWSS eggs for at least 7 days after oviposition. We also made observations on the relative phenology of parasite species and investigated their mating and oviposition behavior. Parasitism on GWSS egg masses by these two parasitoids has been studied on egg masses of different age during the development period range (9-10 days at summer temperatures). Parasitoids were able to effectively parasitize 100% of the egg masses laid on holly plant leaves from the first day after being laid until the day before sharpshooter emergence. Emergence of parasitoids from parasitized egg masses 8-10 days old was somehow limited due to leaves hardening because of age.

Beginning in July at Monticello Florida, 99% of field collected GWSS egg masses on 5-gallon potted experimental plants of Hollies, *Pyracantha*, *Euonymus*, Citrus, Bradford pear, and Crape Myrtle were found with 100% of eggs parasitized. Parasitism rate remained at this high rate for the rest of the season until the end of the oviposition period, which in Monticello was the second week of November.

Parasitoid distribution and survival may be affected by available alternative food sources such as nectar and pollen, especially at times during low host density of hosts. We initiated experiments looking at overwintering behavior and requirements of GWSS and its parasitoids. In a greenhouse kept with open screens allowing parasitoids in an out but without heat of any kind, the last GWSS egg masses where oviposited the second week of November when parasitoids were still active. Those egg masses were a total of seven, 4 and 3 oviposited respectively on two 1-gallon holly plants (*Ilex opaca* var. *cornuta*). Overwintering *Gonatocerus* sp. parasitoids emerged from 2 of the 7 egg masses on February 11th, 2001 and GWSS nymphs emerged from the other 5 egg masses. This indicates for the first time in the U.S. that GWSS as eggs and *Gonatocerus* sp. within parasitized egg masses can overwinter at north Florida winter temperatures.

Laboratory experiments showed that adult parasitoids preferred whitefly honeydew to 50% honey solution and that parasitoids fed honeydew provided from excised leaves with live whiteflies lived twice as long as those fed simple honey solution. Parasitoids may need food sources to sustain them and the low numbers of parasitoids and parasitism of GWSS eggs observed in California in early season may be related to this need. Perhaps parasitoid abundance could be enhanced by providing alternative food sources. It was also found at Monticello, Florida, that an earwig, *Doru taeniatum,* and tree cricket species appear to be important predators of GWSS egg masses particularly early in the season. Because California has native earwig species and early season impact from parasitoids is low, earwigs deserve more research attention for their potential impact on GWSS.