VIRULENCE ANALYSIS OF THE PIERCE'S DISEASE AGENT *XYLELLA FASTIDIOSA*

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INTRODUCTION

The glassy-winged sharpshooter, *Homolodisca coagulata*, spreads the causative agent of Pierce's disease, the bacterium *Xylella fastidiosa* (*Xf*). Depending on whether the glassy-winged sharpshooter can establish itself in Northern California, Pierce's disease may represent a multi-billion dollar threat to the grape and wine industry and the associated tourist trade. The symptoms of Pierce's disease include a yellowing and gradual necrosis (scorching) of grapevine leaf edges, stunting of cane growth, and, particularly in the spring for vines infected for two or more seasons, inter-veinal chlorosis of leaves (Hewitt, 1970). (Lee et al., 1982) reported that detached grape leaves from grape cultivars that are particularly sensitive to Pierce's disease, showed typical marginal yellowing and scorching in less that 12 hr after petiole uptake of cell-free washing from *Xf* cells grown on agar plates. The activity did not survive autoclaving or multiple freezing and thawing and was not inactivated by incubation with proteinase K. No activity was observed for washings of uninoculated agar plates.

Applying the conditions of (Lee et al., 1982), (Goodwin et al., 1988) also observed phytotoxicity after petiole uptake of cell-free washing, but from both *Xf*-populated and uninoculated agar plates. Further, (Goodwin et al., 1988) found a correlation between increased midday stomatal resistance and symptom development on leaf margins and an approximately six-fold increase in leaf proline content (fresh-weight basis) associated with Pierce's disease. They discounted phytotoxins as significant contributors to Pierce's disease symptoms and state that "The biophysical and biochemical changes observed for diseased vines indicate that marginal leaf necrosis occurs when water stress develops. Diseased leaves are apparently water stressed because of vascular dysfunction which, when prolonged, may result in accelerated leaf senescence." (Goodwin et al., 1988) also found that higher stomatal resistance was associated with spring inter-veinal chlorosis but was not as pronounced as the stomatal resistance increase observed for leaves showing symptoms first at the margin.

OBJECTIVE

The objective of this project is to identify gene product(s) and gene(s) of *Xf* that contribute to its virulence.

RESULTS AND CONCLUSIONS

Experimental infection of grape to induce disease generally requires weeks or months to the appearance of symptoms. We wondered whether other plants might exhibit more rapid symptom development upon exposure to *Xf*, particularly to *Xf* infiltrated directly into the leaf lamella. A survey of plant species and lines commonly used as experimental hosts for plant pathogens revealed that *Chenopodium quinoa* (Cq) developed chlorosis corresponding to the infiltrated area within 48 hr of infiltration of 10^8 to 10^6 cfu/ml suspensions of *Xf* cells. Light microscope cytological studies carried out by Prof. Judy Jernstedt, UC Davis Agronomy and Range Sciences Department, revealed that the observed chlorosis of Cq leaves infiltrated with *Xf* is the result of chlorophyll loss from chloroplasts in all photosynthetic cell types without other observable effect. Xanthomonads are considered to be closely related to *Xylella* spp., and *Xanthomonas campestris* pv. *Vesicatoria* and some other, but not all, *Xanthomonas* spp. induce a similar chlorosis in Cq leaves. *Xanthomonas campestris* pv. *campestris* induced necrosis 3-4 days after infiltration. Currently we are testing the effects of petiole-feeding of grape leaves with *Xf* and fractions (see below) derived from *Xf*.

Chlorosis development varied significantly from leaf to leaf on a Cq plant and from plant to plant. However, comparisons of infiltrated opposite leaf halves appear to provide valid measures of the relative potency of *Xf*-derived preparations and forms the basis for a semi-quantitative assay used here. *Xf* cells from liquid culture had a slightly greater specific chlorosis-inducing (CI) activity than *Xf* cells from agar plates. However, the yield of *Xf* cells from plates was greater, and results

reported here are from plate-derived *Xf* cells. The CI activity was associated with cells, not washings of cells. Heating *Xf* cells at 100°C for 6 min slightly enhanced the CI activity. Treatment with sodium dodecyl sulfate (SDS) at 25°C or 65°C did not destroy the CI activity. Most proteins will not survive in active form after treatment at high temperatures or with SDS, but the CI activity was greatly reduced or destroyed by incubation with protease K, trypsin or chymotrypsin. Treatment with acetic acid or chloroform also was inactivating, but incubation with periodate, lysozyme, or ethanol did not reduce CI activity.

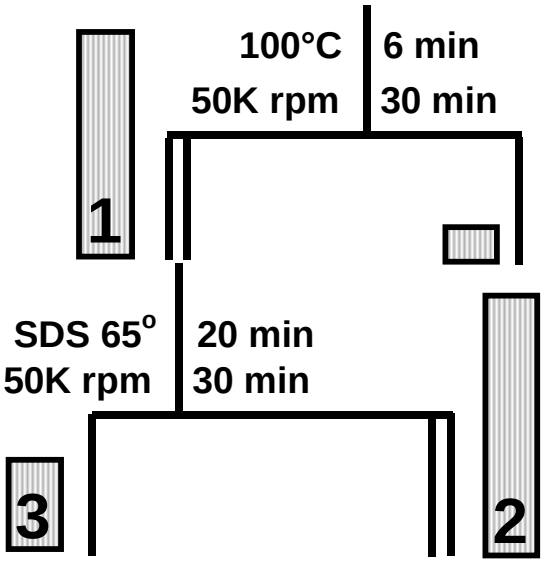
The results described above are consistent with an essential role for an unusually stable protein in whatever constitutes CI activity. Fig. 1 presents an analysis of proteins collected as a precipitate after treatment of *Xf* cells at 100°C or with SDS at 25°C and centrifugation at 50,000 rpm for 30 min. Fig. 2 is a flow chart showing treatment at 100°C and collection of insoluble material, which then was treated with SDS at 65°C. A single vertical line represents supernatant; a double vertical line represents precipitate. Bars show the relative CI activity of four fractions. Even after treatment with SDS at elevated temperature, most of the material remained insoluble. However, exposure to SDS and mercaptoethanol solubilized proteins for gel electrophoresis. As indicated by Fig. 1 and Fig. 3, elevated temperature and SDS removed the bulk of the protein without destruction of CI activity. Lane numbers in Fig. 3 correspond to sample numbers in Fig. 2. Current effort is aimed at correlating CI activity with a specific protein band(s), identification of the corresponding protein, with aid of *Xf* genome sequences (Simpson et al., 2000), and testing of *Xf*-derived, CI fractions for their effects on grape.

Fig. 1

1. *Xf* cells
2. 100°, 6 min
3. SDS RT, 30 min

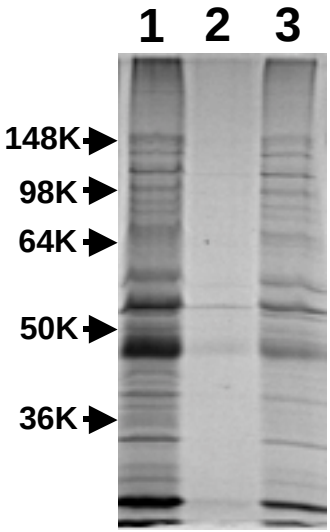
12.5% SDS-PAGE, Coomassie blue stain

Fig. 2



1. *Xf* cells, 100 deg 6 min, high speed pellet
2. #1 + SDS 65 deg 20 min, high speed pellet
3. #1 + SDS 65 deg 20 min, high speed supernatant

Fig. 3



12.5% SDS-PAGE Coomassie blue stain

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EFFECTS ON VERTEBRATES OF RIPARIAN WOODLAND MANAGEMENT FOR CONTROL OF PIERCE'S DISEASE

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INTRODUCTION

It is vital to the wine industry to be aware of the potential effects of control programs for disease, insects and other pests that might affect wildlife in riparian zones, as the health of these zones is important to properly functioning ecosystems as well as to the public and regulatory agencies. This study undertook to evaluate the effects on a variety of vertebrates of the removal of vegetation as well as planting of non-disease reservoir plants in riparian zones to control or reduce the incidence of Pierce's disease. Studies were initiated in 1997 with the establishment of eight plots, three on Conn Creek (open, managed, and not managed), and three on the Napa River (Ecological Reserve, managed, and not managed) in Napa County, and two (managed and not managed) on Maacama Creek in Sonoma County.

OBJECTIVES

1. Determine what influence habitat manipulation for Pierce's disease control will have on birds, mammals, reptiles and amphibians in riparian zones.

RESULTS AND CONCLUSIONS

Five sampling procedures were used per plot as follows:

i. Two Trailmaster bait stations once per month for 7 days (1997-1999)
ii. Fifteen Sherman live traps for small mammals one night per month (1997-1999)
iii. Three point census plots for birds once per week during the breeding season (spring-early summer 1998 and 1999)
iv. Eight nesting boxes for birds (Dahlsten and Copper 1979) checked weekly during the breeding season (1998 and 1999) and
v. Six 4-square foot reptile boards checked approximately twice per month (1998 season).

The bait stations showed the most common mammal to be the opossum, followed by squirrels, raccoons, rats, and foxes. Larger mammal activity was lowest in the open grassy plot, and highest in the Maacama Creek managed plot and the Ecological Reserve unmanaged plot. There were no significant differences in totals of all species between the managed and unmanaged pairs of plots. The most common mammal in the live traps was the deer mouse, *Peromyscus* spp. Activity was highest in the open unmanaged plot and lowest in the Conn Creek unmanaged plot, with no significant differences between the managed and unmanaged paired plots. The most common species in order were swallow species, European starling and American robin. Out of 66 and 72 species identified on the study plots during 1998 and 1999 respectively, with plots averaging about 38 species each. Numbers of birds did not vary significantly between treated and untreated plot pairs except for one pair in 1999 (plots 2 and 3). Numbers decreased significantly overall from 1998 and 1999, especially

in plots 4 and 5. Eight species of birds used the nest boxes and the highest occupancy rate was in the unmanaged area on the Napa River, followed by the managed area at Conn Creek. The Reserve area had the lowest occupancy rate. No patterns were evident in use between pairs of managed and unmanaged plots. The reptile boards were used by four species of lizards, two species of snakes, the Pacific tree frog, western toad, and a salamander. The most common organism was the western fence lizard. The highest use was in the managed plot on Conn Creek; with the other plots being similar to each other, with no significant differences in total numbers between managed and unmanaged plot pairs.

Analyses of our data support the hypothesis that the vegetation management used in this study does not affect numbers of mammals, birds, and reptiles significantly in these riparian areas. Significant overstory differences (plots without many large trees vs. all others with large trees) do show effects with some mammals and birds, and there are also some differences between the Napa Valley plots and the Sonoma plots.

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SEARCH FOR AND COLLECT EGG PARASITOIDS OF GLASSY-WINGED SHARPSHOOTER IN SOUTHEASTERN USA AND NORTHEASTERN MEXICO

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INTRODUCTION

Observations in northeastern Mexico and Texas in 1999 and 2000 revealed presence of *Homalodisca coagulata* (Say) (GWSS) there, but in extremely low densities during winter and spring months (Coronado-Blanco et al. 2000; Triapitsyn & Phillips 2000; Triapitsyn et al. in press). Almost all egg masses of GWSS and other related sharpshooters, such as *Oncometopia* spp., were heavily parasitized. The climate in central part of Tamaulipas, Mexico, is very similar to one in the valleys of southern California, particularly Temecula. Earlier surveys in Florida and Louisiana revealed several species of GWSS egg parasitoids there; some of those species do not occur in California (Triapitsyn et al. 1998).

As a result of the collections made in northeastern Mexico during 2000, quarantine and insectary colonies of three species, *Gonatocerus ashmeadi* Girault, *G. morrilli* (Howard), and *G. triguttatus* Girault (Mymaridae), were established in UC Riverside quarantine and insectary (Morgan et al. 2000; Triapitsyn et al. in press) and later propagated and released against GWSS in California by the California Department of Food and Agriculture (CDFA). Unfortunately, due to unavailability of the host material (i.e., GWSS eggs) during the winter of 2000-2001, the California colonies of *G. triguttatus* and other exotic parasitoids were discontinued.

OBJECTIVES

1. Recollect the target species of GWSS egg parasitoids, particularly *G. triguttatus* Girault, in northeastern Mexico and Texas, clear them through UCR quarantine, and reestablish their cultures (maintained by our cooperators from the CDFA) in southern California for a large-scale classical biological control program against GWSS in California.

2. Search for and collect additional biological control agents, among others *Gonatocerus atriclavus* Girault, *G. fasciatus* Girault, and *Ufens spiritus* Girault (Trichogrammatidae, also known as *Zagella* sp., see Triapitsyn et al. 1998), in the home range of GWSS (southeastern USA and northeastern Mexico) for introduction into California and establishing cultures in UCR quarantine.

RESULTS AND CONCLUSIONS

Two trips were made to date: 1) to Nuevo León, Tamaulipas, San Luis Potosí and Veracruz, Mexico, in February 2001 (D. Morgan, S. Myartseva, G. Simmons, S. Triapitsyn, and D. Yanega) and 2) to Florida, from Miami to Monticello and Quincy (M. Hoddle and S. Triapitsyn) and College Station, Texas (S. Triapitsyn and V. Berezovskiy) in August 2001. One more trip to Tamaulipas is planned for November 2001. In addition, several shipments of adult egg parasitoids, mainly *G. triguttatus* and *G. morrilli*, were sent to UCR quarantine by W. Jones (material reared from GWSS egg masses in and in the vicinity of Weslaco, TX) as well as extensive material from the exploratory trip to northeastern Mexico in March 2001 by D. Morgan and Ch. Pickett (CDFA) was processed in UCR quarantine. Getting the material from Tamaulipas and Texas early in the season helped in re-establishing a colony of *G. triguttatus* first in UCR quarantine and insectary and then at the CDFA facility in Riverside (D. Morgan).

The following species of egg parasitoids were collected during 2001 and propagated at UCR (if applicable):

Genus and species of egg parasitoid	Originally from: (State, location)	Original host	Propagated on GWSS at UCR quarantine (yes/no)
Gonatocerus ashmeadi	Tamaulipas	*H. coagulata*, *Oncometopia clarior*	Yes
	Texas (Weslaco)	*H. coagulata*	Yes
	Florida	*H. coagulata*	Yes
Gonatocerus atriclavus	San Luis Potosí, Tamaulipas, Veracruz	*Oncometopia* spp. including *O. clarior*	No (failed)
Gonatocerus morrilli	Tamaulipas	*Homalodisca* sp. and *Oncometopia* spp.	Yes
	Florida (Apopka)	*Oncometopia nigricans*	Yes
	Florida (Quincy)	*H. coagulata*	Yes
	Texas (Weslaco, College Station)	*H. coagulata*	Yes
Gonatocerus triguttatus	Tamaulipas	*H. coagulata*, *Oncometopia* sp.	Yes
	Texas (Weslaco)	*H. coagulata*	Yes
	Texas (College Station)	*H. coagulata*	Yes
	Florida (Apopka)	*Oncometopia nigricans*	Yes
Acmopolynema sema	Florida (Belle Glade)	*Homalodisca insolita*	Yes
Ufens spiritus (=Zagella)	Florida (Quincy)	*H. coagulata*	No (failed)
Ufens n. sp.	Tamaulipas	*Oncometopia* sp.	No (failed)

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EGG AGE PREFERENCE AND "WINDOW OF SUSCEPTIBILITY" OF *HOMALODISCA COAGULATA* EGGS TO ATTACK BY *GONATOCERUS ASHMEADI* AND *G. TRIGUTTATUS*

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INTRODUCTION

Understanding the reproductive biology of natural enemies is essential if observed outcomes in the field are to be explained accurately. *Gonatocerus ashmeadi* Girault and *G. triguttatus* Girault both attack glassy-winged sharpshooter (GWSS), *Homalodisca coagulata* [Say] eggs, however it is not known whether they compete for host eggs of the same age, what egg ages are most preferred by females of each species, and which species would be most beneficial for GWSS control. *G. triguttatus* is associated with GWSS in Mexico and Texas and is now being released in limited numbers in Riverside County and elsewhere in California to combat GWSS. *G. ashmeadi* is native to California. Determining the egg age preference of this species will also be valuable in maximizing production for parasitoid releases. Therefore, the following experiments were conducted to determine GWSS egg age preference and the 'window of susceptibility' of eggs to attack by *G. ashmeadi* and *G. triguttatus*. Results presented here are for *G. ashmeadi* and are for egg age categories 1, 5 and 10 days of age. Replicates for 2, 3, 4, 6, 7, 8 and 9 days of age have been set up for both species, however, data is still being collected and analyzed. This will be completed and presented at the December 2001 Pierce's Disease Control Program Symposium.

OBJECTIVES

1. To determine the "window of susceptibility" or vulnerability in days of GWSS eggs to attack by *G. ashmeadi* and *G. triguttatus*.
2. To determine *G. ashmeadi* preference for young, medium and old GWSS eggs.

MATERIALS AND METHODS

For Objective 1, five leaves with 15 GWSS eggs of known age laid on Eureka lemon leaves were placed into a 3 inch ventilated vial cage and exposed to one mated female *G. ashmeadi* (~ 24 hrs of age) for two hours at 25°C. This experiment was replicated ten times for GWSS eggs 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days of age and repeated for *G. triguttatus*. The number of parasitoids that emerged from GWSS egg masses of each age and the sex ratio of emerged progeny was recorded.

Three egg ages (1, 3 and 5 days of age) were selected for Objective 2 to represent young, medium and old GWSS eggs. Ten eggs of each age category were presented simultaneously to each of one female *G. ashmeadi* for one hour. At this time the egg age that the parasitoid was found on was recorded, the parasitoid was removed, and the vials were kept at 25°C. The number of parasitoids that emerged from eggs in each age category was recorded after three weeks.

RESULTS AND CONCLUSIONS

Preliminary results showed that approximately 73% of one-day-old GWSS eggs and 26% of 5-day-old eggs were successfully parasitized by *G. ashmeadi*. Lower parasitism rates of 5 day old eggs may have resulted because GWSS embryos in eggs of this age had developed beyond a stage that most *G. ashmeadi* larvae were able to use after hatching, or higher rates of encapsulation were experienced post-oviposition, or venom injected at time of oviposition was not sufficient to halt GWSS embryo development and facilitate wasp development. Furthermore, Eidmann (1934) suggested that success in parasitizing host eggs that are close to terminal development depends on whether the parasitoid egg is oviposited directly into the

embryo, thereby killing it. If Eidmann (1934) is correct, then 26% of ovipositions by *G. ashmeadi* into GWSS eggs 5 days of age may have killed the embryo allowing the parasitoid to develop successfully.

G. ashmeadi successfully parasitised five day old GWSS eggs which indicates that this species has a wide host age preference. This is favorable for GWSS control because the period of vulnerability to attack by this parasitoid is long, therefore increasing the probability that an egg will be parasitised before it hatches.

Exposure of GWSS eggs 10 days of age to *G. ashmeadi* resulted in 0% parasitism as nymphs were emerging from egg masses of this age at 25ºC. Data for the entire 'window of susceptibility' for *G. ashmeadi* and *G. triguttatus* are still being collected. Once this has been completed the results will be analyzed to determine if different egg age preferences exist between these two species.

Results also showed that regardless of egg age and of how many of the 15 eggs were parasitized, female *G. ashmeadi* generally allocated two males to an egg mass and the remaining progeny were female. This is consistent with Stern and Bowen (1963) fixed sex allocation findings.

Results from Objective 2 showed that parasitism was slightly lower for eggs of 5 days of age compared with 3 days of age (Table 1), thereby supporting the results from Objective 1. However, the number of times the parasitoid was found on each treatment did not significantly differ between the three egg ages (Table 1). Also, parasitism did not significantly differ between eggs of 1 and 5 days of age (Table 1). This may suggest that this parasitoid species does not directly select between host age when all the age categories are able to be parasitised. However, other egg parasitoids, such as *Trichogramma* sp. have been found to distinguish between favorable and unfavorable eggs for parasitism and tend to prefer younger eggs (Pak, 1986; Hintz and Andow, 1990; Godin and Boivin, 1994). Therefore, this choice experiment will be repeated next season using the three egg age categories 1, 4 and 8 days of age to determine whether *G. ashmeadi* can differentiate between favorable and unfavorable eggs for parasitism.

Table 1. Percentage parasitism of 1, 3 and 5 days of age GWSS eggs by G. ashmeadi.

Egg age treatment	*Parasitism*	*Number of times the parasitoid was found on each treatment after one hour*
1	22.7%	5
3	27.9%	6
5	20.2%	5

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ELEVATION'S EFFECT ON SURVIVAL OF GLASSY-WINGED SHARPSHOOTER IN KERN COUNTY

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INTRODUCTION

The ability of glassy-winged sharpshooter (GWSS) to successfully over-winter within various crops in Kern County, California is of paramount interest to those seeking to control this pest. The eastern edge of the San Joaquin Valley is heavily planted with citrus, a preferred host plant for feeding and oviposition. This belt of host plants located within a suspected over-wintering survival zone could create a "highway" for rapid northern dispersal of GWSS. These extensive citrus plantings are located at various elevations along a thermal belt that experiences warmer winter temperatures than the valley floor. In addition, the valley floor is subjected to fog and continuous temperatures in the range of 3-5° C.

OBJECTIVE

1. Evaluate the effect of elevation and temperature on the winter survival of glassy-winged sharpshooter in Kern County.

RESULTS

GWSS were placed into replicated field cages at ten different elevations on December 8, 2000. Field cages were framed with PVC pipe (40"x40"x60") and covered with a fine mesh material, trade name Econet B. Four cages were erected at each site. Three different host plants were placed inside each cage at all locations. The type and size of host plants were: parent navel citrus, 5 gal. Established tree, oleander, 1 gal. and gardenia, 1 gal. Between 200 and 300 GWSS field collected adults were placed inside each cage. No attempt was made to determine age or sex of GWSS at the time of collection. The primary objective is to determine if there are areas where GWSS will not survive the winter in Kern County. Temperature and humidity measurements are collected at fifteen-minute intervals for each site. The test will run until the end of April or until new egg masses are found within the cages.

Table 1 summarizes the 10 test site locations, elevation above sea level and hours at or below 0°C. All cages and the plant material within them were retrieved from the ten test locations. All plant and cage material was inspected for GWSS egg masses, nymphs and adults and observations on GWSS survival were made. The results about the survival of GWSS in the cages are provided in Table 2.

Observations and counts suggest that GWSS may experience severe over-wintering mortality at the lower elevation test sites. It is not known what influences test techniques might have had on GWSS survival. Improvements to the study could include the use of larger potted plants. Beefwood trees (*Casuarina* spp.*)* could make an excellent addition to the host plants within any future GWSS study. This study will be repeated in 2001-02 with additional emphasis on elevations between 350-500 feet.

Table 1. Test site locations and elevations.

Location	*Elevation*	*Hours 0-°C**
UCCE Farm Advisors Office, S. Mount Vernon Ave.	371'.	140
Fairfax and Panama Rd.	404'.	191
Panama Rd.	449'.	180.5
AEWSD Ponds, Tejon Hwy.	497'.	37
USDA trailer, Vineland and Edison.	514'.	58.3
Rockpile Rd. and Panama Rd.	579'.	70.8
AEWSD plant, Redbank and Malaga.	606'.	19.0
AEWSD plant, Neumarkle Rd.	712'.	98.5
AEWSD plant, General Beale Rd.	834'.	2.8
Nuemarkle and Bena Rd.	902'.	9.8

Hours at 0° C from Dec. 4, 2000 - April 2, 2001

Table 2. Observations on GWSS survival in the cages.

Site #	*Observation*
1	No GWSS eggs, nymphs or adults found in any of the test cages. Last recorded sighting of a live GWSS in these cages, 1/02/01.
2	No GWSS eggs, nymphs or adults found within any test cage. Last recorded sighting of a live GWSS in these cages, 1/18/01.
3	No GWSS eggs, nymphs or adults found within any test cage. Last recorded sighting of a live GWSS in these cages, 1/18/01.
4	GWSS egg masses found on citrus in cages No. 3 & 4, 4/02/01. No GWSS found in cages No. 1 & 2.
5	GWSS egg masses found on citrus in cages No. 1 & 3, and one adult GWSS female recovered in cage No. 3, 4/12/01. No GWSS Found in cages No. 2 or 4.
6	No GWSS eggs, nymphs or adults found in any of the test cages. Last recorded sighting of a live GWSS in these cages, 2/28/01. Site over-sprayed by a dormant application to almond trees next to test cages.
7	GWSS egg masses found on citrus in all four cages. GWSS egg masses found on gardenias in cages No. 1,2 & 4. Two females and one male GWSS found in cage No. 1.
8	GWSS egg masses found on citrus in cages No. 1 & 3. No GWSS found in cages No. 2 &4.
9	No GWSS eggs, nymphs or adults found within any test cage. Last recorded sighting of a live GWSS in these cages, 3/14/01. This site was exposed to the East non-cropped desert area
10	GWSS egg masses found on citrus in cages No. 1, 2 & 3. GWSS egg mass found on gardenia in cage No. 2. Three females and one male found in cage No.1. Two females and one male found in cage No. 3. No GWSS found in cage # 4.

*****All cages were inoculated with 200 – 300 field collected GWSS adults on Dec. 8, 2000.

EVOLUTION AND HISTORICAL ECOLOGY OF THE PROCONIINI SHARPSHOOTERS

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INTRODUCTION

The tribes Proconiini and Cicadellini, commonly referred to as sharpshooters (Cicadellinae sensu Young 1968), together form the largest group of xylem-feeding leafhoppers and include most of the known vectors of xylem-born phytopathogenic organisms. Proconiini is a strictly New World group with most of its diversity confined to the tropical regions. Among ca. 350 described proconiine species, currently grouped into 56 genera, less than 10% (4 genera) occur north of Mexico, and only one genus (*Cuerna*) has substantially radiated in the temperate parts of the U.S. and Canada. In spite of its largely tropical distribution, the group is of significant economic importance in the U.S. because several *Homalodisca*, *Oncometopia*, and *Cuerna* species are able to transmit *Xylella fastidiosa (Xf)* (Nielson 1968), and two of them recently invaded new areas in the southern U.S. (Pollard et al. 1959; Sorensen & Gill 1996). Species of *Oncometopia* and *Acrogonia* are also principal vectors of *Xf* causing the citrus variegated chlorosis in South America (Gravena et al. 1998). Except for a few economically important species, the data on the ecology and bionomics of proconiines are virtually absent, although this picture is beginning to change. In particular, recent studies suggest that in at least 13 proconiine genera females display a unique type of maternal care by powdering their egg nests with brochosomes, specific Malpighian tubule products unique to leafhoppers (Rakitov 1999, 2000, and unpublished; Hix 2001). Although the exact adaptive significance of this behavior is not yet clear, its occurrence in several of the most speciose proconiine genera, including *Homalodisca*, *Oncometopia*, and *Acrogonia* , which contain principal vectors of *Xf*, suggests that its advantages may have facilitated speciation and contributed to the pest status of some species, perhaps by increasing their capability to colonize a wide range of vegetation. Building a robust estimate of the evolutionary relationships among Proconiini is important to provide a framework in which ecological and bionomical features, and the patterns of distribution of the group can be better understood. In the absence of fossil evidence this can be achieved by the phylogenetic analysis of the distribution of morphological, molecular, and other characters among modern species. Unfortunately, the only such analysis of Proconiini performed so far (Mejdalani 2000) focused on only one group of presumably related genera and therefore has not completely elucidated the relationships within the tribe.

OBJECTIVES

1. To assess phylogenetic relationships among proconiine leafhoppers, using morphological and DNA sequence data.
2. To study the evolution of “egg nest powdering”, and its role in the diversification of the tribe.

RESULTS AND CONCLUSIONS

Our preliminary morphology- and sequence-based analyses both indicated that Proconiini are derived from within Cicadellini. This result is consistent with biogeographical evidence, which suggests that the tribe is younger than the Cosmopolitan Cicadellini and apparently arose in South America after its separation from Africa (Nielson & Knight 2000). A much broader sampling is, however, needed to pinpoint a particular cicadelline group that might have given rise to Proconiini. Our morphological dataset strongly indicated that the Neotropical genera *Pamplona* and *Pamplonoidea*, previously placed in Cicadellini (Young 1977), and a small Neotropical group previously classified as subfamily Phereurhininae (Kramer 1976) should be placed in Proconiini, and that *Homalodisca*, as currently defined, includes certain species that should be placed in other genera. These results, which emphasize the need of a reclassification of the Proconiini, were largely congruent with an estimate based on the mitochondrial DNA sequences (Figure 1). Because of the small size of the molecular dataset, which included representatives of only 27% of the proconiine genera, the presented tree should be interpreted with caution. The analysis grouped the species into two major lineages, one of which included all of the known vectors of *Xf* The habit of coating egg nests with brochosomes appears to have arisen early in the evolution of this lineage; its origin and the vector status of some of the lineage’s members may both be related to certain changes in the ecology of the group. This behavior has apparently been independently lost in several taxa. Comparative studies of the egg-laying ecology in such taxa (e.g., *Cuerna*, *Phera*) and other proconiines may shed light onto its adaptive significance. Our future goals include building a more robust phylogenetic estimate by increasing both the taxon and character sampling, collecting more data on the ecology and bionomics of the group through field work and collaboration with sharpshooter researchers

in other countries, and performing more diverse analyses of the historical ecology, bionomics, and patterns of distribution of the Proconiini.

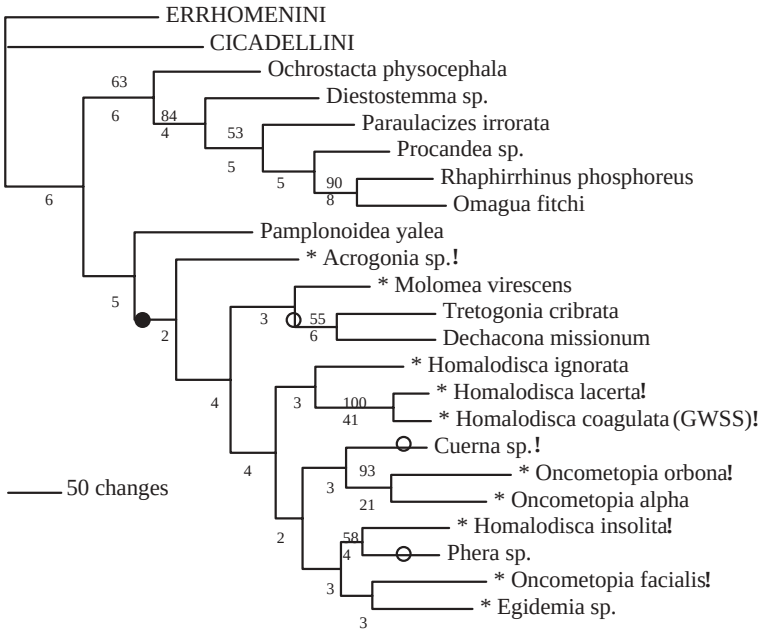

Figure 1. Preliminary estimate of phylogenetic relationships among the species of Proconiini, based on combined partial 12S, 16S, COI, and COII mitochondrial DNA sequences (ca 2000 base pairs) obtained for 21 proconiine and 2 outgroup species. The tree shown is a single maximum parsimonious tree; the length of branches is proportional to the number of nucleotide changes along each branch. The numbers above and under branches are bootstrap scores (shown only if greater than 50%) and decay indices, respectively; higher values indicate better support for the clade. Asterisks (*) indicate species using brochosomes in egg laying. The gain of this trait is shown by a closed circle, and three independent losses by open circles. Exclamation points (**!**) indicate known vectors of *Xylella fastidiosa*.

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