DEVELOPING A STABLE CLASSIFICATION OF THE GLASSY-WINGED SHARPSHOOTER GENUS *HOMALODISCA*

Project Leader:
Stuart H. McKamey
Systematic Entomology Laboratory
PSI, Agricultural Research Service
U. S. Department of Agriculture
c/o National Museum of Natural History
Smithsonian Institution
Washington, DC 20560-0168

Cooperators:
Roman Rakitov
Center for Biodiversity
Illinois Natural History Survey
Champaign, IL 61820

Andrew Hicks
University of Colorado Museum
Boulder, CO 80309-0218

Gustavo Moya Raygoza
Universidad de Guadalajara
Guadalajara, Mexico

Daniela Takiya
Center for Biodiversity
Illinois Natural History Survey
607 E. Peabody Dr.
Champaign, IL 61820

Carolina Godoy
Instituto Nacional de Biodiversidad
Santo Domingo de Heredia
Heredia, Costa Rica

Marco Gaiani
Facultad de Agrícola
Universidad Central de Venezuela
Maracay, Venezuela

Reporting Period: The results reported here are from work conducted from December 20, 2002 to October 15, 2003.

INTRODUCTION

The glassy-winged sharpshooter (GWSS), the leafhopper principally responsible for the spread of Pierce's disease on grape in California, is the species *Homalodisca coagulata*. This special capacity relates to the tissue upon which all sharpshooters (leafhopper subfamily Cicadellinae) feed: xylem, and the invasive status of the GWSS in California. It is noteworthy that of the 19 species in the genus, only one other species occurs in California and 18 species occur outside the USA (6 of these also occur in the USA). The genus is common in Mexico and also occurs southward through Central America, northern South America, and southeastern Brazil and Paraguay. That is, most species of *Homalodisca*, were they to reach California, have a destructive potential equal to the GWSS regarding the grape industry. The genus *Homalodisca* contains two other species that are already known to vector phytopathogens and it is practically expected that all species in the genus have the capacity to be, or become, serious vectors. Clearly, in a situation like this, we need to be clear about which species we are studying. The genus has never been revised.

Words are the tools of efficient communication and taxonomy is the vocabulary of species. By linking information to genus and species names, a classification of species becomes at once a very efficient system for storage and retrieval of information, and hence for meaningful communication, and a predictive tool, provided that classification is sound. Linking that information to species names that may be based on misidentifications, or belong to entirely different genera, will only add confusion to vector studies. In order to communicate effectively about the GWSS and its congeners, it is essential that everybody use the same names for the same species.

Access to all information on any group of organisms, including *Homalodisca*, is severely impeded by arbitrary generic limits, multiple names for some species and no name for others, or the absence of authoritative identification tools, or all three factors. The status of *Homalodisca* in this regard is below acceptable levels for a group of such economic importance.

OBJECTIVES

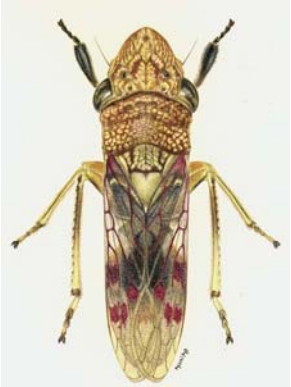
Broadly, the objective of the proposed research is to stabilize the classification of the genus *Homalodisca* so that all other information gathered (host plants, ecology, physiology, genomics, etc., which are all identified as priorities in the PD research program) can be linked to the correct names for meaningful communication. This will be accomplished through three major objectives:

1. Establish the limits of the genus *Homalodisca* though comparison to closely related genera, and the limits of all species in the genus, determine their valid names, and describe new species as necessary.
2. Characterization of brochosome structure and related behavior to allow identification of egg masses and females for most species.
3. Provide authoritative and electronically accessible identification aids and distribution data for all species, in addition to a hardcopy publication of the *Homalodisca* revision.

Also important for a revision is determining the relationship of *Homalodisca* to closely related genera. This is presently being addressed by Ph.D. student and proposal cooperator Daniela Takiya, with outside funding for four years and is consequently not a major objective of this project.

RESULTS AND CONCLUSIONS

Objective 1. About 1,000 specimens of *Homalodisca* have been examined in detail and locality data has been extracted and converted to decimal degree geographic coordinates for about 1,500 specimens. Morphological comparison among populations and species of *Homalodisca* has yielded a preliminary data matrix that will, when completed, be used to estimate relationships among species. At present, the species *H. insolita* appears to warrant its own genus, and the description of the type species of *Homalodisca* appears to refer to the genus *Propetes*, and not congeneric with other species currently in *Homalodisca*. As international rules of nomenclature require that a genus name must remain linked to its type species, there is a possibility that a new generic name may be needed to refer to the glassy-winged sharpshooter and its congeners. In June 2002, the first expedition of the project was conducted, in Costa Rica. It was an enormous success, yielding two species of *Homalodisca* with host and distribution data, oviposition behavior (recorded on film), and samples of egg masses for both.



This has enabled accurate illustration of color patterns (Figures 1, 2) which darken over time in preserved specimens, fresh material for molecular analyses of *Homalodisca*, and more progress in Objective 2.

Figure 1. *Homalodisca icthyocephala*, colors based on freshly captured specimen in Costa Rica.

Additionally, fresh specimens, hosts, oviposition behavior, egg samples, and two likely parasitoids were obtained for several close relatives of *Homalodisca*. These additional collections will facilitate development of a more predictive classification and improve quarantine efforts.

Figure 2. *Homalodisca insolita*, colors based on freshly captured specimen in Costa Rica.

Objective 2. In *Homalodisca* and a few related genera, females coat egg masses with brochosomes, minute, hydrophobic secretions of the Malphigian tubules that are only found in leafhoppers. These egg-brochosomes differ markedly from cuticular brochosomes and vary in structure among species. Postdoctoral fellow Roman Rakitov has now characterized the brochosomes and brochosome-related behavior of seven species of *Homalodisca* and several of its close relatives, particularly as a result of the team expedition to Costa Rica.

Objective 3. The identification aids and published revision of *Homalodisca* necessarily follow completion of Objectives 1 and 2. An on-line, image-driven key will be produced and placed on the USDA/ARS Systematic Entomology Laboratory server to maximize access and utility. A traditional key to species will accompany the hardcopy generic revision.

Delimitation of the genus and its relationship to other genera presently is being conducted by Ph.D. student Daniela Takiya. Because her analysis is based on molecules and morphology, obtaining fresh specimens of many sharpshooter genera is crucial for this facet of understanding *Homalodisca*. The fieldwork in Costa Rica has provided many specimens for her study, including fresh material of *Homalodisca* and several close relatives, even new species and one new genus in the process of being described.

FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service, Systematic Entomology Laboratory, and the University of California Pierce's Disease Grant Program.

PATTERNS OF *XYLELLA FASTIDIOSA* INFECTION IN PLANTS AND EFFECTS ON ACQUISITION BY INSECT VECTORS

Project Leaders:
Alexander Purcell
ESPM - Division of Insect Biology
201 Wellman Hall
University of California
Berkeley, CA 94720-3112

Steve Lindow
Plant and Molecular Biology
111 Koshland Hall
University of California
Berkeley, CA 94720-3102

Reporting Period: The results reported here are from work conducted from July to September 2003.

ABSTRACT
This study will examine the effect of host plant tolerance on the epidemiology of Pierce's disease (PD), specifically on the ability of the insect vectors to acquire *Xylella fastidiosa* (*Xf)* from plants considered tolerant to PD. The examination of *Xf* population and distribution in tolerant and susceptible plants, and its relationship to xylem anatomy, symptom development, and bacterial acquisition by sharpshooters, may reveal what traits of a plant are important in the overall spread of PD. Since host plant resistance is an important component in the long-term goal of curing PD, it will be important to know how this resistance affects the spread of PD in areas permanently infested with sharpshooter vectors. This research will also assist with the short-term goal of controlling the spread of PD by enabling growers to evaluate grape cultivars on their ability to provide inoculum for vine-to-vine spread of Pierce's disease. We will examine host plant resistance and its effect on the ability of insects to be vectors of Pierce's disease with two tools, GFP-expressing *Xf* and insect transmission of the pathogen. Due to the short time available for experimentation, there are no substantive results available yet.

INTRODUCTION
The GFP-*Xf* retains typical virulence in grape, continually glows green when illuminated with blue light (Figure 1) and allows examination of plant tissues without the extensive fixation required with electron microscopy (8). Alternate hosts of *Xf* selected for their different patterns of *Xf* colonization following vector inoculation, lack of stem lignification, and absence of green autofluorescence under blue light (Figure 2) are annual morning glory (*Ipomoea purpurea* 'Grandpa Ott'), mugwort *(Artemisia douglasiana)*, sunflower (*Helianthus annuus)* and annual bur-sage (*Ambrosia acanthicarpa*) (6, 15). Grape cultivars with varying tolerance to PD selected for evaluation are tolerant 'Sylvaner', moderately susceptible 'Cabernet Sauvignon' and highly susceptible 'Chardonnay' cultivars of *Vitis vinifera* (11, 12). Both highly efficient blue green sharpshooters (BG) and glassy-winged sharpshooters (GW) will be used to infect plants and assess the efficiency of insect acquisition of *Xf* (1, 5, 10).

Anatomical comparisons between alternate hosts and grape cultivars will include measurements of vessel length and number, and vascular bundle number and distribution based on the techniques of Tyson *et al.* (14), utilizing confocal rather than electron microscopy, and Ewers and Fischer, modified to infuse the pigment via 100kPa pressure applied to the proximal end of the cutting (4, 9). We will evaluate primary vegetative growth rather than secondary xylem due to the difficulties in sectioning, culturing from, and feeding BGSS on partially lignified stems. GFP-*Xf* inoculation and colonization of all plants will be measured similarly in all plants: groups of four GFP-*Xf* carrying sharpshooters will inoculate a 3-cm stem section, and the plants will be evaluated for the presence of GFP-*Xf* approximately 8 weeks after inoculation. Colonized vessels will be counted, and populations estimated by culture on PWG media (2, 6).

We will evaluate GFP-*Xf* acquisition by sharpshooters from the alternate hosts and grape cultivars after completion of anatomical characterization. Insects will be caged on GFP-*Xf* inoculated sites for 4 days to acquire the bacteria, and then be placed on another grape seedling for 2 days to determine their acquisition efficiency. Immediately following sharpshooter feeding, the stem site will be examined with confocal microscopy and tested with culture. Three stem cross-sections and three 1-cm long longitudinal sections per site will be sectioned and suspended in 50% glycerol on a depression slide. When illuminated with blue or ultraviolet light, both GFP-*Xf* and the individual vessels are visible, and it is possible to determine the proportion of vessels colonized, the extent of bacterial colonization inside them, and the distribution of colonized bundles. Bacterial populations will be determined from remaining plant material of the same site, and symptom development and severity will be assessed. Data analysis will include comparisons between species for the number, length and distribution of xylem elements. Since acquisition efficiency has been related to bacterial populations (7), we must separate the effects of bacterial distribution and proportion of colonized vessels from the effect of bacterial population. The number of plants we can evaluate in a confocal microscopy session is a limiting factor. A maximum of 90 observations per experiment will allow examination of 5 inoculation sites for each of three species or cultivars. Based on an average of 20.3 bundles per stem (in annual bur-sage), we will be able to detect at least a 20% difference in *Xf* colonization with $\alpha = 0.05$ and $\beta = 0.10$ (13).