SHARPSHOOTER FEEDING BEHAVIOR IN RELATION TO TRANSMISSION OF PIERCE'S DISEASE BACTERIUM

Project Leader:
Elaine Backus
Department of Entomology
University of Missouri
Columbia, MO 65211

Personnel:
Fengming Yan
College of Life Sciences
Peking University
Beijing, China

Javad Habibi
Department of Entomology
University of Missouri
Columbia, MO

William Bennett
Private consultant
744 Elktown Rd.
Otterville, MO

Cooperators:
Matthew Blua
Department of Entomology
University of California
Riverside, CA

Alexander Purcell
Division of Insect Biology
University of California
Berkeley, CA

Edwin Civerolo
USDA-ARS, PWA
Exotic & Invasive Diseases & Pests
Parlier, CA

Reporting Period: The results reported here are from work conducted from November 1, 2001 to October 31, 2002.

INTRODUCTION

Almost nothing is known of the stylet penetration (probing) behaviors of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, and how they interact with populations of Pierce's disease (PD) bacterium, *Xylella fastidiosa*, to facilitate transmission to grapevine. The Backus project is combining the three most successful methods of studying leafhopper feeding (i.e. histology of fed-upon plant tissues, videotaping of feeding on transparent diets, and electropenetration graph [EPG] monitoring) to definitively identify all details of feeding. Both AC and DC EPG monitoring are being performed. All recorded waveforms will be correlated with stylet activities, cell types within the host plant in which activities occur, and presence or movement of *X. fastidiosa* in and out of the stylets. This research will provide crucial baseline information for the present projects of collaborators, as well as the future development of a Stylet Penetration Index for PD inoculation behavior, for screening differences among grapevine varieties and other uses.

OBJECTIVES

1. Identify and quantify all feeding behaviors of GWSS on grapevine, and correlate them with location of mouthparts (stylets) in the plant and presence/ population size of *Xylella fastidiosa* in the foregut.
2. Identify the role of specific stylet activities in *Xylella fastidiosa* transmission, including both the mechanisms of acquisition and inoculation, and their efficiency. Emphasis is on inoculation.
3. Develop a simple, rapid method to assess feeding, or detect the likelihood of *Xylella fastidiosa* transmission (an "inoculation-behavior detection method"), for future studies.

RESULTS AND CONCLUSIONS

We spent the first 4 months of this year completing the purchase of equipment, upgrading facilities, establishing plantings, and hiring personnel, as described in last year's progress report. Unforeseen delays in acquiring a post-doc visa were finally overcome in early February 2003, when Dr. Fengming Yan, Associate Professor, Peking University, arrived. Research was begun in March 2003, and has continued for 8 months. Work this year supported Objectives 1 and 2.

Objective 1

A. Adult GWSS were collected on citrus in Riverside, California by Cooperator Matt Blua, who express-mailed them to Missouri every 2-4 weeks from mid-February to mid-October 2002. Sharpshooters were maintained on chrysanthemum and basil under quarantine, but conditioned for 48 hours on grapevine, cv. 'Cabernet Sauvignon' (from FPMS, UC Davis) prior to testing on grape.

B. For Experiment 1, Yan and Backus EPG-monitored a total of 242 male and female sharpshooters feeding on chrysanthemum or grapevine, for access periods ranging from 4 to 20 hrs. Both AC and DC EPG monitors were used, each with separate insects. We used these results to identify, characterize and label waveform phases, families and types, after the now-standard conventions used for EPG (Reese et al. 2000, Cline and Backus 2002). The most common categories of AC waveforms and their characterizations are described in Table 1; representative appearances are pictured in Figure 1A and B. In general, AC and DC recordings looked quite different, but were dividable into the same 3 phases, designated: pathway, ingestion and interruption (Figure 1A). Both AC and DC waveforms were very complicated at the most expanded (fine-structure) level of characterization (termed waveform types) (Figure 1B). In the interest of time, we characterized the DC waveforms only to phase, while concentrating on more in-depth characterization of the AC waveform types, until we perfect the AC-DC correlation monitor (see D below). Preliminary analysis suggests that there are no differences in AC waveform types between males and females, or among insects feeding on chrysanthemum or grapevine. Selected traces will be quantified and descriptive statistics applied for a preliminary publication.

C. In September 2002, Bennett completed the building and, with Backus, the testing of a prototype AC-DC correlation monitor, whose design was based on suggestions kindly provided by W.F. Tjallingii (of Wageningen University, The Netherlands; pers. comm. and Tjallingii 2000), with modifications by Bennett (ms. in prep). This monitor allowed, for the first time ever, display of two simultaneous signals from the same feeding insect, one AC and the other DC. Its only drawback was that the two views were not absolutely identical to those of normal AC and DC monitors. However, they were very similar and interpretable, and further minor adjustments may make them closer to normal. This new monitor will facilitate future correlation of DC waveforms with existing AC waveform categories.

D. For Experiment 2, Yan and Backus developed protocols to: 1) rapidly terminate the feeding of wired sharpshooters, to produce short EPG excerpts ending in a certain waveform type, and 2) mark the feeding site on grapevine petiole, for excision of the plant tissue containing the salivary sheath. Yan then performed 98 such waveform terminations, with matching excised petioles fixed for histological examination of sheaths. Habibi is preparing, sectioning and examining these tissues, locating salivary sheaths and producing digital micrographs. To date, a total of 36 salivary sheaths have been correlated with the 6 presently identified AC waveform types (Table 1), i.e. 4 to 7 for each type. Results from preliminary analysis by Backus and Yan are summarized in Table 1. In short, we found that sheaths from pathway waveforms indeed lie along a path to the xylem. However, not all sheaths from ingestion-containing excerpts terminate in mature xylem elements. Some also terminate in proto-xylem or xylem sclerenchyma, as well as pith cells or interfascicular bundle sheath cells. In several cases of multi-branched sheaths, we could assign some branches to a certain waveform event by comparing degree of hollowness of each branch. Preliminary analysis suggests that the shortest-duration events occur in immature or non-xylem cells, while the longest-duration events occur in mature tracheary elements. However, this conclusion must be verified.

E. For Experiment 3, Yan used AC EPG and videomicrography to record sharpshooter feeding on Parafilm sachets containing expressed grape xylem sap (provided by Collaborator Purcell). Preliminary analysis shows that sharpshooters performed all of the pathway waveform types on such diet. However, ingestion waveforms were abnormal and their duration was very brief; even the hungriest insects terminated probing after only a few minutes. Sheath salivation was easily visible, although protocol modification will be necessary before watery salivation can be detected. Further recordings and frame-by-frame analysis will allow many correlations of waveform fine structure with stylet activities.

Objective 2

A. For Experiment 4, Yan and Habibi used the same waveform excerpting and plant techniques for a study correlating waveforms and salivary sheathes with inoculation of *Xylella* to healthy grapevine. In addition, all sharpshooter heads were excised and fixed for later scanning EM, to allow additional correlation with size and appearance of *Xylella* colonies inside the precibarium and cibarium. Eight treatments were performed, using a 2x4 factorial, randomized complete block design with 10 replicates of each treatment. The treatments were composed of two waveform excerpt treatments ([1] pathway only, or [2] pathway + 1 hr of ingestion [including any interruptions]) and four *Xylella* detection methods ([1 and 2] plants held in the greenhouse for 6 weeks, then fed-upon tissues tested via PCR [by Collaborator Civerolo] or bacterial culturing [by Collaborator Purcell]; [3] plants held for three months, then assessed for PD symptom development; or [4] plants held for 5 d, then the fed-upon petiole histologically prepared for immunocytochemical detection of both salivary sheaths and *Xylella*). This experiment is still in progress.

REFERENCES

Cline, A.R. and E.A. Backus. 2002. Correlations among AC electronic monitoring waveforms, body postures and stylet penetration behaviors of *Lygus hesperus* (Heteroptera: Miridae) on cotton. Environ. Entomol. 31: 538-549.

Reese, J.C., W.F. Tjallingii, M. van Helden and E. Prado. 2000. Waveform comparisons among AC and DC electronic monitoring systems for aphid (Homoptera: Aphididae) feeding behavior. *In* G. P. Walker & E. A. Backus [eds]. *Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior*. Thomas Say Publications in Entomology: Proceedings.

Tjallingii, W.F. 2000. Comparisons of AC and DC systems for electronic monitoring of stylet penetration activities by homopterans. *In* G. P. Walker & E. A. Backus [eds]. *Principles and Applications of Electronic Monitoring and Other Techniques in the Study of Homopteran Feeding Behavior*. Thomas Say Publications in Entomology: Proceedings.

FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

Table 1. Proposed categories, their characteristics and meanings, for the most common AC waveforms of the GWSS.

Phase Name	Waveform Name	Waveform Characteristics	Proposed Biological Meaning	
			Plant Tissue/Cell	Insect Activity
Non-probing	Z	Irregular, small waveforms; amplitude and frequency vary	Plant surface	Walking on plant surface, moving around, labial dabbing
Pathway	A1	Highest amplitude, ascending waveform at beginning of probe w/ or w/o spikes at the top	Parenchyma or bundle sheath	Breakage of plant surface, secretion of salivary sheath and/or watery saliva
	A2	Medium amplitude, declining slope; irregular high frequency	Parenchyma or bundle sheath	Lengthening and/or hardening of salivary sheath
	A3	Medium amplitude, relatively flat irregular high frequency	Parench., bundle sheath or xylem	Further sheath salivation
	B	Regular, high frequency, short (4~5 s), with distinct phrases	Vascular or inter-fascicular tissue	Stylet tip fluttering? possibly w/ sheath salivation?
Ingestion	C (to be subdivided)	Regular, low frequency with distinct phrases	Usually xylem, but sometimes pith	Ingestion (watery excretory droplets correlated)
Interruption	N	Irregular, appears A-like, but occurs during C; ave. duration 16 sec	Vascular or inter-fascicular tissue	Salivary sheath extension or branching

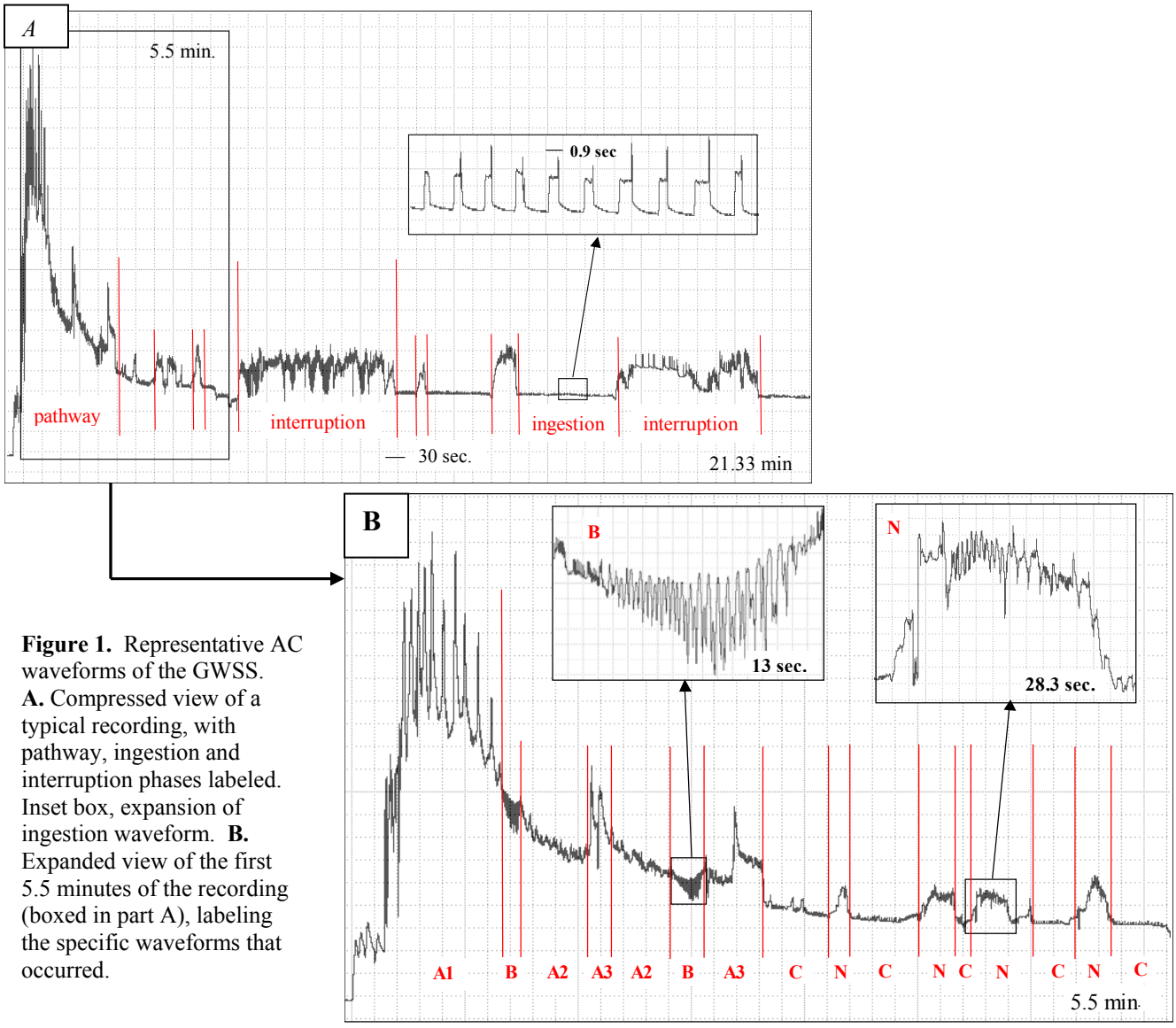

Figure 1. Representative AC waveforms of the GWSS. **A.** Compressed view of a typical recording, with pathway, ingestion and interruption phases labeled. Inset box, expansion of ingestion waveform. **B.** Expanded view of the first 5.5 minutes of the recording (boxed in part A), labeling the specific waveforms that occurred.