EXPLORATION FOR FACULTATIVE ENDOSYMBIONTS OF SHARPSHOOTERS

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INTRODUCTION

Improved biological control of the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata*, in California has been a major objective of research attempts to lower the incidence of this vector of *Xylella fastidiodsa (Xf)*, a bacterial pathogen of grapes, almonds, alfalfa (in California), citrus, coffee (in Brazil) and a variety of other plants (Purcell and Feil 2001). So far, the most promising biological control approaches have been to seek new parasitic wasp species that attack the eggs of GWSS and to discover ways to enhance their effectiveness (Triapitsyn et al. 1998). Pathogens of GWSS have not been employed to date largely because none are known, although recent research is directed towards discovering viruses of GWSS.

Endosymbiotic bacterial associates of leafhoppers are little-understood and unexploited components of their biology that we believe could make a significant contribution to control of these pests. The gravity of the threat posed by GWSS justifies research into this unexplored aspect of its biology. Biological control by itself is unlikely to provide a solution to the GWSS/PD dilemma, but hopefully will serve as a cornerstone to an integrated approach by lowering populations of GWSS to the point where combinations of other control methods such as insecticides, repellents, and habitat management can lower the numbers of infective GWSS in affected crops to manageable levels.

Of particular interest to us are bacterial associates that are facultative (also referred to as "secondary"), i.e., that occur in some individuals or populations but not others, and that could be introduced into, or augmented in pest populations. We use the term symbiont here in the biological sense of 'living together" and do not imply mutual benefit (Douglas 1994). Facultative bacterial associates have been described in a variety of Homoptera including leafhoppers (Swezy and Severin 1930, Schwemmler 1974, McCoy et al. 1978, Purcell et al. 1986). The only leafhopper facultative symbiont studied in some depth is BEV, a bacterium that occurs in *Euscelidius variegatus* in France, but apparently not in California (Purcell et al. 1986). Uninfected females of *E. variegatus* inoculated with cultures of BEV transmitted the bacteria transovarially to their offspring. Subsequently, infected leafhoppers had drastically reduced fecundity (by 80%), slowed development (double the normal development time), and increased mortality, relative to uninfected controls (Purcell et al. 1986, Purcell and Suslow 1987). They also transmitted phytoplasma bacteria at drastically reduced rates, but we do not anticipate this would occur with *Xf*.

It is clear from our studies of facultative bacteria in aphids (Chen et al. 2000, Montllor et al. 2002) as well as from the study of BEV, that endosymbiotic associations are complex and have critically important effects, both positive and negative, on the physiology, population biology and vector potential of their hosts. Any component of leafhopper behavior, physiology, or ecology that affects their ability to vector plant pathogens can have major implications for the spread of plant diseases. The most likely impacts that facultative symbionts might have on the control of GWSS involve their potential ability to:

- decrease populations of GWSS to lower equilibrium levels (may be temperature dependent)
- change (+ or -) the rate of successful parasitization by biological control agents
- decrease GWSS fecundity
- facilitate production of GWSS parasitoids (if discovered to be limited by bacterial symbionts)

OBJECTIVES

1. Survey glassy-winged sharpshooter and other sharpshooters in California and the Southeastern U.S. for facultative bacterial endosymbionts.
2. Determine by DNA sequencing the identity of any bacteria discovered.
3. Depending on type of microorganism and relative frequency in surveyed insects, select candidate symbionts to (a) attempt to culture, (b) determine whether they can be transmitted by injection of hemolymph from infected to uninfected GWSS or to other sharpshooter species, (c) determine whether they are transovarially transmitted, (d) determine whether they can be horizontally transmitted through plants and (e) determine whether any are beneficial or pathogenic to GWSS in terms of life history traits (growth, fecundity, longevity, parasitism).

RESULTS AND CONCLUSIONS

Glassy-winged sharpshooters have been collected in Louisiana, Florida and California (two locations) in spring and summer 2002. Four other species of sharpshooters have also been collected in California in summer 2002. DNA has been extracted from hemolymph samples of 170 insects. To date, the 16s ribosomal DNA from 88 of these samples has been amplified with universal eubacterial primers. About 60% of the samples contained enough amplified DNA to detect by electrophoresis, indicating either that some insects do not have detectable bacteria in the hemolymph, or that the hemolymph sample collected was too small. Eubacterial DNA from 25 individual GWSS from Florida, Louisiana and California was digested with the endonuclease Hinf l as an initial screen for differences in the amplified bacterial DNA. These digests have been analyzed by electrophoresis, yielding distinct band patterns. Five different digest patterns have been found to date in these GWSS, and at least two band patterns have been documented from each locality. There appears to be overlap in band patterns among the three localities (i.e., common patterns in insects from the three areas), though this is not yet certain. The presence of a variety of band patterns indicates that several types of bacteria are present in our samples. Although great care has been taken to avoid contamination of hemolymph samples, we do not yet know whether some of the bacterial DNA in our samples might come from an exogenous source. We have also extracted DNA from the primary symbionts of GWSS, which occur in structures called bacteriomes, and which can easily be dissected out of the insect, and from BEV-infected leafhoppers, for comparative purposes.

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