

## CITRUS AND COFFEE STRAINS OF *XYLELLA FASTIDIOSA* INDUCE PIERCE'S DISEASE IN GRAPEVINE

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*Xylella fastidiosa* causes citrus variegated chlorosis disease in Brazil and Pierce's disease of grapevines in the United States. Both of these diseases cause significant production problems in the respective industries. The recent establishment of the glassy-winged sharpshooter in California has radically increased the threat posed by Pierce's disease to California viticulture. Populations of this insect reach very high levels in citrus groves in California, and move from the orchards into the vineyards, where they acquire inoculum and spread Pierce's disease in the vineyards. We now show that strains of *Xylella fastidiosa* isolated from diseased citrus and coffee in Brazil can incite symptoms of Pierce's disease after mechanical inoculation into seven commercial *Vitis vinifera* L varieties grown in Brazil and California. Thus any future introduction of the CVC strains of *X. fastidiosa* into the United States would pose a threat to both the sweet orange and grapevine industries. Previous work has clearly shown that the strains of *X. fastidiosa* isolated from Pierce's disease and citrus variegated chlorosis affected plants are the most distantly related of all strains in the diverse taxon *X. fastidiosa*. The ability of citrus strains of *X. fastidiosa* to incite disease in grapevine is therefore surprising, and creates an experimental system with which to dissect mechanisms used by *X. fastidiosa* in plant colonization and disease development using the full genome sequence data that has recently become available for both the citrus and grapevine strains of this pathogen.

## ULTRASTRUCTURE OF THE BACTERIOME-ASSOCIATED ENDOSYMBIONTS OF THE GLASSY-WINGED SHARPSHOOTER, *HOMALODISCA COAGULATA* (HOMOPTERA: CICADELLIDAE)

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Light and transmission electron microscopy were used to examine the intracellular endosymbionts of the glassy-winged sharpshooter, *Homalodisca coagulata*. The organisms described are contained within bacteriocytes, forming organs referred to as bacteriomes. In glassy-winged sharpshooter, bacteriomes consisted of paired reddish organs associated with yellow glandular-like structures located in the ventro-lateral anterior portion of the abdomen. Three morphologically distinct bacteria-like organisms were observed. One spherical in shape, one rod shaped, and one larger, highly pleomorphic organism that often appeared elongated. The red portion of the bacteriome appeared to contain only the spherical organisms, while sections of the yellow portion contained all morphological forms. In some cases individual cells in the yellow portion contained more than one type of organism.

## AC AND DC EPG WAVEFORMS OF GLASSY-WINGED SHARPSHOOTERS ON *CHRYSANTHEMUM* AND GRAPE

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Glassy-winged sharpshooter, *Homalodisca coagulata* (Homoptera: Cicadellidae), is a new vector of Pierce's disease bacterium, *Xylella fastidiosa*, threatening production of grape and other fruits in California. Long-term management of this disease will rely on host plant resistance, which will be (could be approached) aided by a complete understanding of the vector's feeding behaviors associated with bacterial transmission. EPG (electrical penetration graph) monitoring is a powerful tool to investigate feeding behaviors of sucking insects on plants. Both AC and DC EPG were used for the first time to study recorded feeding behaviors of sharpshooters on *Chrysanthemum* and grape. The waveforms were categorized into pathway, xylem ingestion and interruption phases, and were correlated and verified with feeding sites on the plant, insect body postures, watery excretory droplets, and histological observation of salivary sheaths within plant tissues.

## USE OF A TRIPARENTAL MATING SYSTEM TO INTRODUCE GREEN FLUORESCENT PROTEIN MARKED TRANSPOSON INSERTIONS IN THE PLANT PATHOGEN *XYLELLA FASTIDIOSA*

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*Xylella fastidiosa* causes many serious diseases of fruit trees in North America, particularly Pierce's Disease of grapevine and 'Phony' disease of peach. In South America the pathogen causes the recently described Citrus Variegated Chlorosis and Coffee Leaf Scorch diseases, both of which are widespread in Brazil. The magnitude of the disease problems caused by this bacterium led to the organization of a consortium in Brazil, which has determined the complete nucleotide sequence of the genome of a citrus strain of the pathogen. Teams in the United States and Brazil have subsequently sequenced the genomes of grapevine, oleander and almond strains of the pathogen. However in order to exploit the genomic sequence data to enable effective disease control, systems for genetic manipulation of the pathogen are necessary, but have thus far been completely lacking. We report the introduction of foreign DNA into a citrus strain of *Xylella fastidiosa* by use of a triparental mating system. With this system we have introduced a mini-Tn5 transposon that encodes a Green Fluorescent Protein (GFP) gene optimized for expression in bacteria. The mini-Tn5 derivative was inserted into different sites of the genome in independent transconjugants as determined by Southern blotting. The position of the insertions was determined by reference to the genomic sequence data. The GFP gene was also expressed well in *Xylella fastidiosa*, and to different levels in different transconjugants. Four independent transconjugants were separately used to inoculate sweet orange and tobacco seedlings. The transconjugants were able to colonize the plants and were subsequently re-isolated from points distal to the inoculation sites. When the relative fluorescence of the transconjugants that had been passed through either tobacco or sweet orange was compared to that of the same transconjugant maintained continuously in vitro, we observed that passage through either plant host significantly increased the level of expression of the GFP. The increased level of expression of GFP was transient, and was lost upon further culture in vitro. We have developed a system for the introduction of marked mutations which will be useful for both in vitro and in planta analysis of gene expression of *Xylella fastidiosa*.