

## Δ9 DESATURASE FROM SHARPSHOOTERS

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### ABSTRACT

Genomics approaches permit the identification of gene functions within insect pests which can then be targeted to reduce fitness. Genomic analyses of several leafhoppers identified the leafhopper delta-9 desaturase motif. Disruption of desaturase by RNAi or other strategies should reduce the biological fitness of leafhoppers, thus aiding efforts to stop the spread of Pierce's disease (PD), and other leafhopper transmitted diseases. The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) and other leafhoppers are vectors of *Xylella fastidiosa*, a xylem-limited bacteria that causes PD in grapevine as well as 'Scorch-like' pathology of other woody agricultural fruit, nut, and ornamentals. Management of PD currently depends heavily upon insecticides to suppress these vector populations. Unfortunately chemical controls are not insect specific, can lead to resistance, and often reduce non-target beneficial organisms. To extend current IPM programs alternatives need to be developed which can work within these programs. Thus, we provide genetic sequences identified with links to food utilization and oviposition, these may serve as RNAi targets to reduce leafhopper fitness.

### LAYPERSON SUMMARY

The identification of genes which are critical for the survival and reproduction of insect pests opens the door for applications of natural insect management based on gene disruption, such as RNA interference (RNAi). We identified the desaturase enzymes in leafhoppers which they need to digest food, store fats, and produce eggs. This is the first step in the development of safe, advanced, management based upon the specific genetics of leafhopper pests. The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) causes economic losses to growers by spreading the plant infecting bacterium, *Xylella fastidiosa*. This bacterium causes Pierce's disease of grapevine, which can stop grape production and kill vines. Therefore, development of effective population management strategies are needed. Insecticides although successful to reduce the economic impact of these insects, ultimately will result in resistance and/or resurgence of insect pests. Thus alternative methods of population suppression are continually being developed. Here we propose the use of gene targeting (desaturases) to reduce the ability of the leafhoppers to grow and reproduce, and describe the desaturase enzymes from leafhoppers.

### INTRODUCTION

Genomic approaches, these data are providing new information on the genetic basis of biology, behavior, and refinement of their phylogenetic classification (Hunter et al. 2003). Three leafhopper species, referred to as sharpshooters (Hemiptera: Cicadellidae) were examined by comparison of the available expressed sequence tags, ESTs, (Hunter datasets, NCBI). The glassy-winged sharpshooter (GWSS; *Homalodisca vitripennis*) is the major vector of *Xylella fastidiosa* (*Xf*) in the southern USA. The plant pathogenic bacterium, *Xf*, has caused economic losses to several agricultural industries in North America and is associated with many plant diseases such as Pierce's disease (PD) of grapevine. The introduction of GWSS into new areas is directly related to increased occurrence of PD in vineyards (Perring et al., 2001). Therefore, the management of PD depends heavily on the ability to reduce the sharpshooter vectors, especially GWSS. Methods of vector manipulation such as chemical control with the use of insecticides are not target specific and lead to problems such as resistance, water contamination, and injury to non-target organisms. Current efforts to develop more benevolent pest management strategies has led to the use of biocontrol agents such as fungi and parasitoids. One of the most exciting developments on the horizon however is in the applications of gene disruption, RNA interference, RNAi. But before RNAi can be implemented gene targets within the sharpshooters need to be identified. Thus, we have started to characterize genes within these sharpshooter species.

### OBJECTIVES

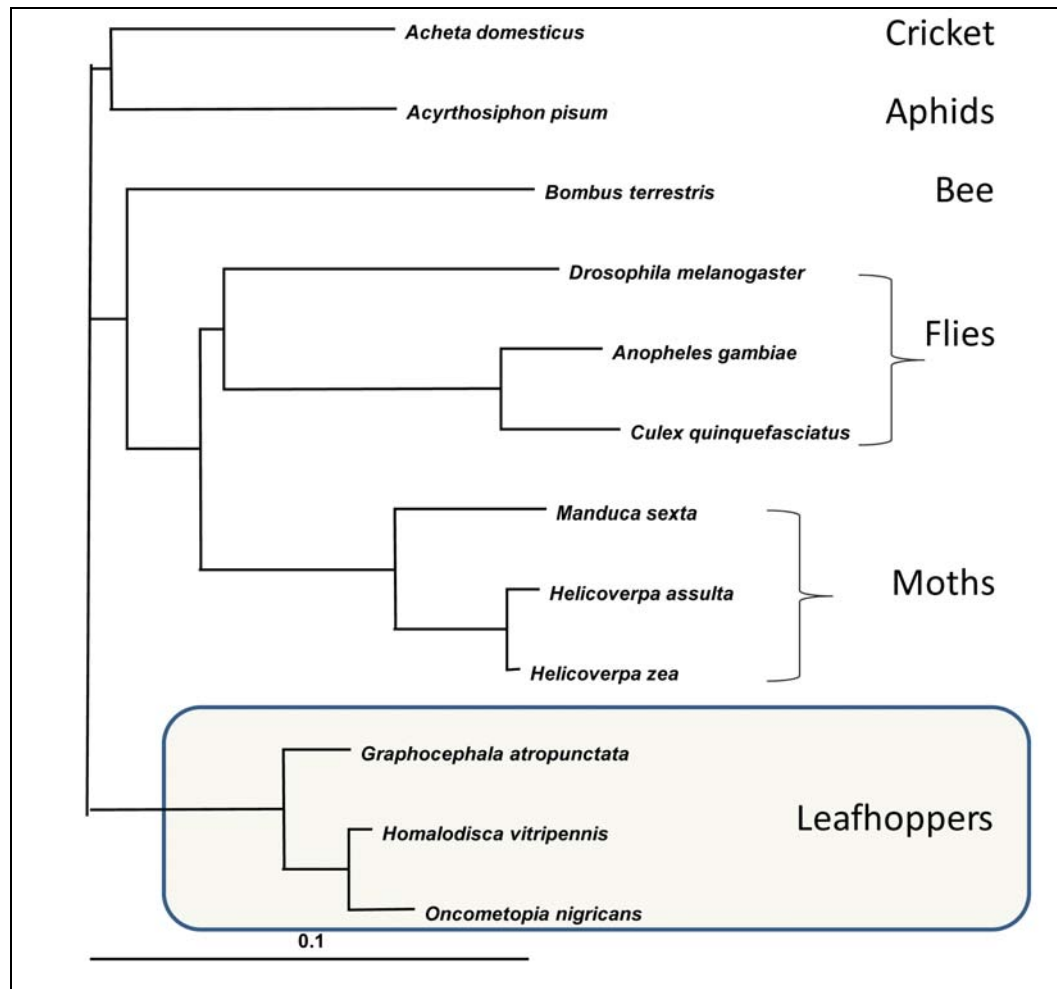
1. Use *in silico* analyses to characterize delta-9 desaturase in sharpshooters.
2. Ultimately develop RNAi to disrupt desaturases in sharpshooters.

## RESULTS AND DISCUSSION

The identified sharpshooter  $\Delta$ -9 desaturases were submitted to NCBI database [gb|AAU95195.1| *O. nigricans*; gb|AAT01079.1| *H. vitripennis*]. Desaturase enzymes have been shown to be highly conserved throughout Eukarya (Fungi, Protists, Plants, and Animals) and function in the processing of lipids by placing double bonds between the adjacent carbons of fatty acids. The  $\Delta$ -9 desaturase also occurs embedded in the membrane of endoplasmic reticulum, ER, and functions as either palmitoyl or stearoyl  $\Delta$ -9 desaturase.

Delta-9 desaturase-1 identified as a palmitoyl desaturase within *H. vitripennis* (Hunter, 2004), and similar  $\Delta$ -9 desaturase were identified within *O. nigricans* and *G. atropunctata* with the specificity of the later two unknown. The sequence analysis of sharpshooter desaturase provides evidence as to the degree of homology between these species.

**Sequence analysis** - Base calling was performed using TraceTuner™ (Paracel, Pasadena, CA) and low-quality bases (quality score <20) were stripped from both ends of each EST. Quality trimming, vector trimming, and sequence fragment alignments were executed using Sequencher™ software (Gene Codes, Ann Arbor, MI). Sequencher contig assembly parameters were set using a minimum overlap of 50 bp and 90% identity. Contigs joined by vector sequence were flagged for possible miss-assembly and manually edited. The  $\Delta$ -9 desaturase sequences obtained from each of the three species were then aligned using Bioedit and conserved domains identified. Further sequence identity was determined based on BLAST similarity searches using the NCBI BLAST server ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) with comparisons made to both non-redundant nucleic acid and protein databases using BLASTN and BLASTX, respectively. Matches with an E-value  $\leq 10$  were considered significant and were classified according to the Gene Ontology (GO) classification system. Translated proteins were analyzed with National Center for Biotechnology Information's BLASTp, Pfam ([www.pfam.org](http://www.pfam.org)), InterProScan ([www.ebi.ac.uk](http://www.ebi.ac.uk)), and Expert Protein Analysis System ([www.expasy.org](http://www.expasy.org)) (**Figure 1**).



**Figure 1.** Tree based on  $\Delta$ 9 desaturase protein sequences of various insect species. Three leafhopper species are shown circled below and accession numbers as follows: *Homalodisca vitripennis* (gi|46561748|gb|AAT01079.1|.) and *Oncometopia nigricans* (gi|53830704|gb|AAU95195.1|.) based on BLAST alignments performed through NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Another partial protein sequence that was identified as *Graphocephala atropunctata* showed 70% coverage and a  $9e^{-109}$  e-value when aligned with *H. vitripennis*. Multiple sequence alignments of predicted desaturase amino acid sequences were performed using ClustalX version 1.81 algorithms (Thompson *et al.*, 1997).

The fact that the largest percentage of ESTs from these leafhoppers, code for lipid metabolism or related processes which should not be surprising considering that lipids and lipid transport play vital roles in insect physiology. The insect cuticle, often accounts for up to 50% of the dry weight of an insect, contains a number of layers containing lipid mixtures with lipid transport systems used for movement into these layers (Lockey 1984). Significant levels of lipids are also deposited into the oocyte during oogenesis to be used as energy for the embryo (Downer and Matthews, 1976) with approximately 30-40% of the dry weight of an egg being lipids (Ziegler and Antwerpen, 2006).

Expression analyses by real-time rtPCR across all life stages resulted in lack of detection of desaturase expression in eggs. However, all other instars 1<sup>st</sup>-5<sup>th</sup> expressed  $\Delta 9$  desaturase showing a significant increase in the 5th instar and adults which correlated to increased feeding on a widening host plant range to complete development, and the formation of new organs (ie. Ovaries). There was no significant difference in expression levels of  $\Delta 9$  desaturase among 1<sup>st</sup>- 4<sup>th</sup> instars. The 5<sup>th</sup> instars showed significantly increased levels of desaturase compared to the previous instars, while adults expressed significantly greater levels from all other life stages. The expression of the GWSS  $\Delta 9$  desaturase for pheromone production is unlikely as there is agreement between the absence of reports of pheromone production in adult GWSS females or males, and the lack of a significant difference in the levels of gene expression between the adult females and males.

## CONCLUSIONS

We isolated sharpshooter sequences with homology to  $\Delta 9$  desaturase 1. Desaturase plays crucial roles in lipid biosynthesis and their levels have been linked to the developmental age of organisms (Aguilar and Mendoza 2006). Desaturases are essential components of a variety of cellular tasks such as catalysis, transmembrane transport and signaling, and are intimately associated with cellular membranes. The majority of analyses performed on insect fatty acyl-CoA desaturases thus far have been focused on Dipteran and Lepidopteran insects leaving scant information from Hemipterans. The deduced amino acid sequence from the GWSS  $\Delta 9$  desaturase shares 77% similarity with the *Acheta domesticus* desaturase and has significant (>50%) similarity with other insect delta-9 desaturases plus having many features of acyl-CoA desaturase to provide evidence towards its function and importance in sharpshooter biology.

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