MICROARRAY ANALYSIS OF GENE EXPRESSION AND DIAPAUSE IN THE GLASSY-WINGED SHARPSHOOTER

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ABSTRACT
The condition of diapause in the glassy-winged sharpshooter, GWSS, *Homalodisca vitripennis*, is poorly understood. Diapause is better known from other, non hemipteran insects. We used oligonucleotide microarrays to address the specificities of transcriptional responses of adult female GWSS, which were in 'diapause', to different lighting regimes. Two of these lighting regimes were known to induce oviposition in diapause females under greenhouse conditions during winter months. Thus we examined female GWSS gene expression during diapause and during the 'breaking' of diapause induced by light. Upon 'breaking' diapause, the females' ovaries became active, produced eggs and females oviposited similar to springtime conditions. The mRNA from 22 individual GWSS adult females was compared. Each individual was hybridized to a single chip. There were six individuals in the control group, and eight individuals in each treatment. Using strict criteria (a twofold change in expression), we determined that a definable number of genes was differentially expressed between the diapause females within the three lighting regimes. Of the 2,126 genes surveyed, five genes showed an increase in expression and two showed a decrease in expression (at least a 2.2-fold change) when comparing the control adult female GWSS to the GWSS exposed to the light treatments. Identification of the genetic basis of diapause will provide genetic targets which may be subjected to 'silencing' or 'down-regulation' by emerging technologies in plant improvement, or through virus delivery, or endophytic bacterial expression systems.

INTRODUCTION
Little is known about the genetic basis of diapause in the GWSS. As winter season approaches the GWSS becomes physiologically suppressed. The ovaries shrink and appear to become non active. These females, which survive the winter months, will emerge in the spring and become active again, thus ovipositing eggs in the early spring which become the next generation. Attempts to mass rear GWSS is difficult and often halts when winter arrived due to the lack of eggs. Some females were noticed to continue to oviposit when under a different light source. Having a condition which manifests a biological response provided a unique opportunity to examine the genetic basis of the effect of light wavelength on the induction of GWSS oviposition.

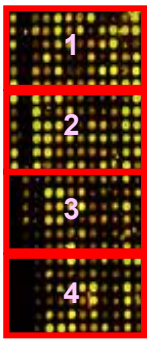
OBJECTIVES
Increase understanding of the genetic basis of diapause in sharpshooters, GWSS. Use of a genomics approach permits examination of leafhopper diapause at the genetic level. Discoveries from these results will advance our understanding of the triggers and biological pathways related to diapause.

RESULTS
The principles of Oligonucleotide and cDNA microarray assay of gene expression: Five genes were identified which responded to Far-Red light (Figures 2, 3). A microarray is a set of short Expressed Sequence Tags (ESTs) made from a cDNA library of a set of known (or partially known) gene loci. In this case the 'Unigene' set used in the production of this array consisted of 2,126 selected genes, produced by Hunter (database NCBI). The data set was mined from four different cDNA libraries produced from 1) adults, 2) 5th instars, 3) midguts, 4) salivary glands. The ESTs produced from previous work are used as a template to prepare smaller oligonucleotides (35 bases long) which are mass produced and then spotted onto a cover-slip-sized glass plate. The GWSS microarray was produced by Combimatrix, Inc., (microarray slide format: 1 X 12,000 features) and was laid out in a repeat of four fields, each with the same 2,126 features to account for within microarray variation (Figure 1). Twelve chips were used for this experiment to evaluate 22 individual GWSS. Four slides in each of three treatments which were stripped and rehybridized with independent GWSS samples to account for between chip differences. A total of 22 individual GWSS were processed and hybridized to the arrays, six in the control, eight in treatment I., eight in treatment II. The control was: Normal grow lights-Phillips fluorescents, two banks of four blacklight 75Watt; Treatment I: was two Sylvania GroLux, 400 Watt, and Treatment II: was one AgroSun Gold Universal 1,000 Watt. Each light was raised or lowered to provide approximately the same light intensity, as determined by a hand held light meter. The mRNA transcriptomes are prepared by extraction from the whole body of a GWSS. Complementary DNA (cDNA) reverse transcripts are prepared and labeled with two different fluorescent dyes. The experimental and control libraries are hybridized to the microarray. Dual-channel laser excitation excites the corresponding dye, which fluoresces proportional to the degree of hybridization that has occurred. Relative gene expression is measured as the ratio of the two fluorescences:

"up-regulation" relative to the control will be visualized as a red "pseudo-colour," "down-regulation" shows as green, and constitutive expression (1:1 versus control) as a neutral black. Intensity of color is proportional to the expression differential.

CONCLUSIONS

The use of Oligonucleotide or cDNA microarrays can measure *life-stage and tissue specific patterns* of gene expression across tens, hundreds, or thousands of genes at a time, thus providing a powerful genomic tool. Genomic approaches to the investigation of diapause in insects have vastly expanded our knowledge about GWSS physiology. Leafhopper genetic analysis will undoubtedly expand and we will make new discoveries involved in the diapause of GWSS. Gene profiling of different leafhopper species, or even across insects, will assist in determining the complex pathways that comprise the response that we call diapause.



Four printed fields each repeats the same 2,126 genetic sequences.

An Oligonucleotide that is 35 bases long is designed to each gene sequence. The oligo is then mass synthesized and spotted onto the chip.

Total RNA is isolated from one insect, and labeled for each chip Hybridization.

Within chip variation, and between chip variation is controlled for, with significance being 2.5 fold difference of Increase or decrease of detection.

Figure 1. Microarray chip layout design, 1x12K

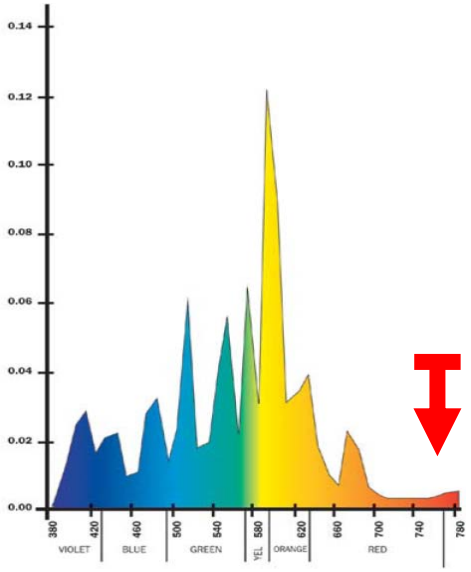


Figure 2. Wavelength spectrum chart from 1000 Watt bulb, Agrosun Gold Universal. Microarray analysis determined five genes were up regulated in response to stimulation to light in the Far Red spectrum (RED Arrow). GWSS which were in 'diapause' show a condition of reduced ovaries and no egg production. A light regime of two weeks, with an additional two weeks to start egg production produced females which were able to lay eggs and which had active, enlarged, ovaries.

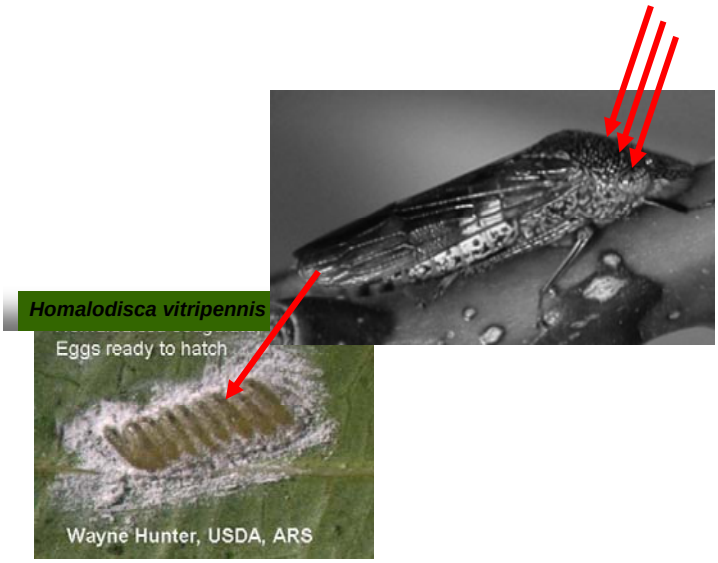


Figure 3. GWSS in diapause have reduced ovaries, and no egg production. In the spring the ovaries become revitalized and egg production starts again. Light and temperature affects the insect brain and physiology.

To identify the wavelength of light which may be involved in inducing the 'breaking' of diapause we evaluated gene expression in GWSS that were in diapause, after they were exposed to three different light sources which had different light spectra. We identified five genes which were significantly upregulated during GWSS exposure to far red light. These GWSS developed enlarged, active ovaries.

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