

Interim Progress Report for CDFA agreement number 11-0145-SA

**Title of Project**           The endocrine system of GWSS, a viable insecticide target

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**Time period covered**        April 1, 2012 to June 30, 2012

**Introduction**

Insect development is precisely regulated by the relative titers of juvenile hormone (JH) and molting hormones. JHs are a family of sesquiterpenoids that regulate reproduction, behavior, polyphenisms, development, and other key biological events in insects (reviewed in Riddiford, 2008). Minor disruption of an insect's hemolymph JH levels can result in insect death or dramatic alterations in insect development. Juvenile hormone analog (JHA) insecticides are green compounds that selectively target the insect endocrine system by mimicking the biological action of JH (reviewed in Dhadialla et al., 2005; Henrick, 2007). When pest insects are exposed to JHAs at a time during development when JH titer is normally undetectable, abnormal nymphal-pupal development and/or death is induced. Abnormal developmental morphologies, similar to those induced by JHAs are also induced by inhibiting an esterase that selectively metabolizes JH (Abdel-Aal and Hammock, 1985). Inhibition of the JH-selective esterase (JHE) putatively results in JH titers that are not below the threshold required for normal development. Similarly, we hypothesize that inhibition of another JH-metabolizing enzyme called JH epoxide hydrolase (JHEH) will also result in the induction of abnormal nymphal-pupal development and/or death of GWSS.

In this project we are testing the efficacy of commercially available JHAs against GWSS eggs and nymphs. We are also attempting to characterize the JHEH of GWSS, an enzyme that metabolizes the epoxide moiety that is found on all known JHs. The gene that encodes this enzyme, *jeh*, could have potential as a target for gene silencing-based control of GWSS. In terms of mode of action, the effects of JHA application, and JHEH and/or JHE knockdown by gene silencing are similar in that both approaches can enhance "JH action".

**List of objectives**

- I. Investigate the delayed effects of low dose JHA insecticide exposure
  - A. Determine sublethal dose in eggs and 1<sup>st</sup> instar nymphs
  - B. Evaluate delayed effects of sublethal exposure on egg development
  - C. Evaluate delayed effects of sublethal exposure on nymph development
- II. Characterize recombinant JHEH from GWSS
  - A. Clone full-length *jeh* gene of GWSS
  - B. Biochemically characterize recombinant JHEH
  - C. Screen JHA insecticides for JHEH inhibitory activity

**Description of activities conducted to accomplish each objective**

Objective II. Characterize recombinant JHEH from GWSS.

During previous reporting periods, we characterized both JHEH and JHE enzyme activity levels in the hemolymph of 5<sup>th</sup> instar GWSS nymphs. The peak activity of JHEH (9.3 ± 1.7 pmol of JH diol formed per min per ml of hemolymph) was found at day 6 of the 5<sup>th</sup> instar. During the current reporting period total RNA was isolated from nymphs at day 3, 4, and 5 or the 5<sup>th</sup> instar. These RNAs were used to generate first strand cDNAs. The first strand cDNAs were then used as template for random amplified cDNA end (RACE)-based attempts to identify the 3'-end and 3'-UTR of a potentially JHEH-encoding cDNA. These attempts were unsuccessful. Subsequently, total RNA was isolated from older 5<sup>th</sup> instars (days 6, 8, or 10), and these RNAs were used to generate first strand cDNAs for 3'-RACE. This 3'-RACE approach was successful. Use of template first strand cDNAs generated from 5<sup>th</sup> instar, day 10, total RNAs identified a putative JHEH-coding sequence. On the basis of this sequence, several gene-specific, nested primers were designed for 5'-RACE. The 5'-RACE identified the 5'-UTR and 5'-end of a potentially JHEH-encoding cDNA.

On the basis of the 5'- and 3'-RACE results two primers were designed to amplify a full-length cDNA encoding JHEH. Using these primers, we were able to PCR-amplify a full-length cDNA, *hovi-jheh*, that potentially encoded JHEH. In order to express a recombinant protein encoded by *hovi-jheh*, the full-length cDNA was subcloned into the transfer vector plasmid pAcUW21. We have transfected the resulting plasmid with parental baculovirus DNAs, and are currently in the process of isolating a recombinant baculovirus capable of expressing recombinant GWSS JHEH for further characterization.

## Summary of accomplishments and results

We have identified a putative full-length JHEH-encoding cDNA (i.e., *hovi-jheh*) from 5<sup>th</sup> instar, day 10, nymphs. Nucleotide (lower case text) and deduced amino acid (upper case text) sequences of *hovi-jheh* are shown in Fig. 1. The 5'- and 3'-UTR sequences, and coding sequence of *hovi-jheh* are 174, 101, and 1,374 nts-long, respectively. Predicted amino acid residues that form the putative catalytic triad (D-227, H-432, and E-405), lid domain (Y-300 and Y-375), and oxyanion hole (HGWP, residues 152-155) are shown in bold text. The asterisk indicates a stop codon (TAG). A putative membrane anchor domain (residues 2-24) that was predicted by SOSUI software is shown in italic text. A predicted cleavage and polyadenylation specificity factor (CPSF) complex binding site is underlined.

## Publications produced and pending, and presentations made

No peer-reviewed publications or presentation have been made during the reporting period.

Figure 1

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catttatatcagagtgagtcaggctgcttgtagttacagagtgaagctgagacag
at aagi cacat cccact ct gtt c ggt t c t g g g c g g a a g t t t g t t g a a t a c a t t c t c t t t
at cct agct caaacct g t t g t t a c c t g g t g t c c a a t a a a t t t t t a g e t a t t g e c c a
at gat caagggt g t g t g g t c a g t g e t a g t g g t g a t g t c t c e a g t t g e a t t g g g e t a
M I K G V L L S V L V V S A V A L G L
t a c a t a g a c t a c a a c a a t g e t t t c a c t g g t a c a g c c c a t c a c a a t a a t g e a a t g g t g g
Y I D Y N N A F T G T A P S P I M Q W W
g g a g a g g t a a a c c t a g a t t a g a t g a t g g c a t c a t c a a a c c a t t c a a a a t c a a c t t t c c c
G E G K P R L D D G I I K P F K I N F P
a a a g a g c t t c t g g a t g a t t t g e a t c a t c g t c a g e a a c a c t e g e c c t t c c a c c a c c a
K E L L D D L H H R L S N T R P F H P P
c t a g a c g a t g t g g g t t c c a g t a t g g a t t a a c a c a g a g t t c t c g a a c t g c t c g g a c
L D D V G F Q Y G F N T E F L Q T V L D
t t t t g g a g a a c g a a a t a c t c c t g g a g g a a c g t c a a a a t t a c t t g a a c t c e c t g e c t c a a
F W R T K Y S W E E R Q N Y L N S L P Q
t a c a c a a t g g a a a c t c a a g g c c t g e a g a t c a c t t c a t c c a t g t a a a a c a a a a a a t g c a
Y T M E T Q G L Q I H F I H V K P K N A
a a a g g a t t a c a t g t a t a t c c a c t c e t g e t a g t c c a t g g t t g g c c a g g t t c t g t g a g a g a a
K G L H V Y P L L L V H G W P G S V R E
t t t t a t g a t a t c a t c c c t c t g e t g a c t a c t c e a a g a a g a a g a a g a t t t g t g t t g a g
F Y D I I P L L T T P R E G Q D F V F E
g t a g t t g e c c a t c a c t g e t g e t a t g a t t t c a c a g g t a c a a c c e g a c a t g g t c t t
V V A P S L P G Y G F S Q A T T R H G L
g g a c a g c c c a g g t c g c c g t t g t c t t g e c a c t c t a t a a g c a a c t g g g t c a c a a a g g
G P A Q V A V V L R T L M K Q L G H K R
t a c t a t g t c c a g g c g g a g a t t g g g c a g e a t c a t t g c a c t c t g e t t g e a c a g a t g a c c
Y Y V Q G G D W G S I I V T L L A Q M T
c g t g g t g a t a g g a t t c t t g g a c t t c a c a a a a t a t g e g t a t g t t a t g e c a a c t a c t g g a
R G D R I L G L H T N M A Y V M P T T G
a t g a t g g c t g g c a a a t c a t c g c a g t t g g t a t c c a a g t g t a t t g e t g a t g a a c a g a a
M M A W Q I I G S W Y P S V I G D E T E
c a g a a g t a t g t t a t c c a a t g t c t g a t c a c t a c t c c t t t t t g e t a g a g a a t c t g g a t
Q K Y V Y P M S D H Y S F L L E E S G Y
t t g e a t c t g e a a g e t a c c a a g e c t g a c a c a a t a g e t g t a g c t t t g a g a g a t t c a c c g a t g
L H L Q A T K P D T I G V A L R D S P M
g e g e t g e c t g e t t a c a t c t t g g a g a a g t t c t c a a c t t g g a c t g a t c g t t c a t g g a a g a t
G L A A Y I L E K F S T W T D R S W K S
c t c a a g g a t g a g a g t c t t c t t a a a a a g t t c a a g c t g g a a g c t a t t t g g a c a a t g t c a t g
L K D G G L L K K F K L E A L L D N V M
a t c t a c t g g a t g a c a g g c a c c a t c a c t a c t c e a t c e a t g e t c t g t a c a g t g a a c a c t t c a a c
I Y W M T G T I T T S M R L Y S E H F N
a g g a c c a a t c g a g c a t t g g g c t t g c a c c a g c a c a g t a a a a g t a c c t a c g g c g a t a g c t
R T H R A L G F D Q A P V K V P T A I A
c g t t t c c c c a a t g a g e t c g c c a a t c a g e c t c g a t g e t t t t a g a g a a a a g t t c a c g a a t
R F P N E L A N Q P R C F L E K K F T N
t t g g t g c a a g t a a c a g a c a t c c c t a c a g a a g g t c a c t t t g a g c c t t t g a g g a a c c t a a a
L V Q V T D M P T G G H F A A F E P K
c t g t t g g c t g a t g a t a t t g g g a t t t g t c a g e c t c t g c a t c a c a a t c c a c c t a g e c c
L L A D D I W D F V S A L H H K S T *
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a c t g e c c a a g c c t a c a c a a g t g e t a a c a c a a a t a a t t a a t c a a a g a c t g a c t t t t a
a t a t a c a t t t c t t a c c a a t a a a g c c t t a t a c t t t g t a a a a a a a a a a a a a a a

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### **Research relevance statement**

The insect endocrine system is a highly selective and highly sensitive target for insect control and for reducing vector competence. The overall goal of our project is to study and exploit targets within the endocrine system of GWSS that can be used to control GWSS or reduce its ability to spread Pierce's Disease. We are taking two complementary approaches to accomplish this goal. Our first approach is to determine the efficacy and effects of juvenile hormone analog (JHA) insecticides against GWSS. A key objective of this approach is to quantify the minimum level of JHA insecticide that can efficiently reduce the emergence of nymphs from eggs and keep nymphal insects in the nymphal stage. The results of this direct approach will have near-term applicability since the JHA insecticides that we are testing are US-EPA registered and commercially available. Our second approach involves the identification and characterization of genes that are unique to the GWSS endocrine system that metabolize a key insect hormone called JH. The objective of this approach is to characterize and evaluate these genes as potential targets for gene knockdown.

### **Layperson summary of project accomplishments**

During the reporting period we have identified and cloned the sequence of a gene that likely encodes an important enzyme that is involved in the regulation of GWSS development. This enzyme may be a target of novel RNA-based strategies to control GWSS development.

### **Status of funds**

As of July 24, 2012, \$16,317 remains for the original funding amount.

### **Summary and status of intellectual property associated with project**

No issues associated with intellectual property have been generated with the project.

### **Literature cited**

- Abdel-Aal, Y.A.I., Hammock, B.D., 1985. 3-Octylthio-1,1,1-trifluoro-2-propanone, a high affinity and slow binding inhibitor of juvenile hormone esterase from *Trichoplusia ni* (Hubner). *Insect Biochem.*, 15: 111-122.
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