THE ENDOCRINE SYSTEM OF THE GLASSY-WINGED SHARPSHOOTER, A VIABLE INSECTICIDE TARGET

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ABSTRACT
Minor disruption of the endocrine system can result in dramatic changes in insect development. Juvenile hormone analog (JHA) insecticides are green compounds that selectively disrupt the insect endocrine system. In this project we are testing the efficacy of JHAs against glassy-winged sharpshooter (GWSS) eggs, nymphs, and adults. We are also evaluating the potential of juvenile hormone esterase (JHE)- and juvenile hormone epoxide hydrolase (JHEH)-encoding genes as a target for gene silencing-based control of GWSS. In terms of mode of action, the effects of JHA application, and JHE and/or JHEH knockdown are similar in that both approaches can enhance "JH action" during periods of developmental when endogenous levels of JH are exceptionally low.

LAYPERSON SUMMARY
The overall goal of this project is to study and exploit targets within the endocrine system of the glassy-winged sharpshooter (GWSS) that can be used to reduce the spread of Pierce's disease. We are taking two complementary approaches in this project. The first is a direct approach in which the efficacy of juvenile hormone analog (JHA) insecticides such as fenoxycarb and pyriproxyfen are being tested against GWSS eggs, nymphs, and adults. The objective of this direct approach is to identify JHAs that can efficiently (*i*) reduce the emergence of nymphs from eggs, (*ii*) keep nymphal insects in the nymphal stage, and/or (*iii*) reduce egg viability prior to oviposition. The results of the JHA efficacy trials will have near-term applicability since the JHAs that we are testing are US-EPA registered and commercially available. The second approach involves the identification and characterization of GWSS genes that metabolize a key hormone in insects called JH. The objective of this approach is to evaluate these genes as a potential target for gene silencing.

INTRODUCTION
Insect development is precisely regulated by the relative titers of juvenile hormone (JH) and molting hormones (i.e., ecdysteroids). JHs form a family of sesquiterpenoids (**Figure 1A**) that regulate key biological events in insects including reproduction, behavior, polyphenisms, and development (reviewed in (Riddiford, 2008). Minor disruption of an insect's hemolymph JH levels can result in dramatic alterations its development. Juvenile hormone analog (JHA) insecticides are green compounds that selectively target the insect endocrine system (reviewed in (Dhadialla et al., 2005; Henrick, 2007). JHAs such as methoprene, fenoxycarb, and pyriproxyfen (**Figure 1B**) are US EPA-registered compounds that are commonly used to control mosquitoes, fleas, whiteflies, ants, and other insect pests. JHAs function as mimics (both structural and biological) of juvenile hormone (JH), a key insect developmental hormone. 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. Similar abnormal development morphology can also be induced by the inhibition of a JH-selective esterase (JHE) with a chemical inhibitor such as OTFP (Abdel-Aal and Hammock, 1985). Inhibition of JHE putatively results in JH titers that are not below the threshold required for normal development. In this project we are attempting to identify and clone the *jhe* gene and related JH epoxide hydrolase, *jheh*, gene. The potential of these genes as a target for gene silencing (so that glassy-winged sharpshooter (GWSS) development can be disrupted) will also be evaluated.

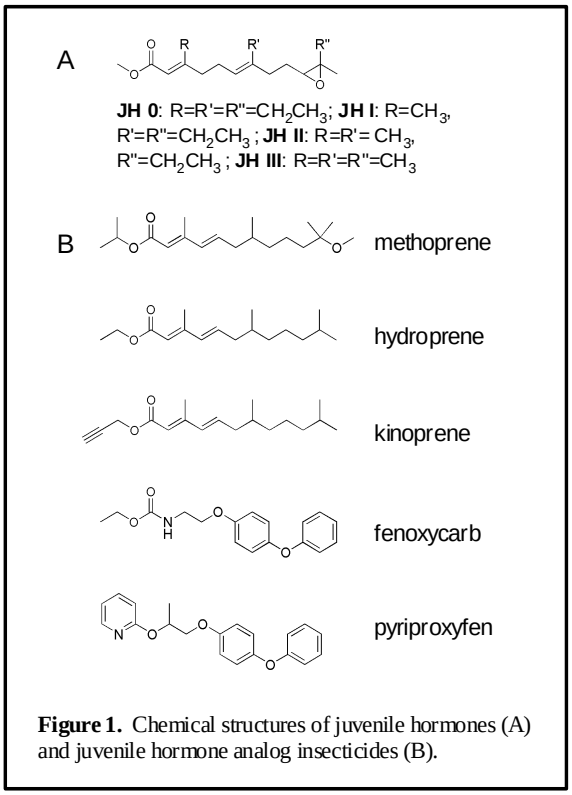

Figure 1. Chemical structures of juvenile hormones (A) and juvenile hormone analog insecticides (B).

OBJECTIVES

1. Evaluate the efficacy of JHA insecticides
    A. Determine median lethal dose and effective time in nymphs
    B. Determine median lethal dose in eggs
    C. Evaluate ovicidal effects following treatment of adult females
2. Characterize authentic JH esterase (JHE) activity in GWSS
    A. Quantify authentic JHE activity in 5th instar nymphs
    B. Evaluate the ability of OTFP to inhibit JHE activity in nymphs
3. Isolate *jhe* gene and characterize recombinant JHE protein
    A. Isolate JHE coding sequence from GWSS
    B. Express and biochemically characterize recombinant GWSS JHE
    C. Test the efficacy of an RNAi approach to silence GWSS *jhe*

RESULTS AND DISCUSSION

I. Evaluate the efficacy of JHA insecticides

A laboratory colony of GWSS has been established at the UC Davis Contained Research Facility with the help of our cooperator Bryce W. Falk. The GWSS colony is reared in an environmental growth chamber (Percival Scientific, Perry, IA) set for a 14 h:10 h light:dark cycle at 24ºC and 70% relative humidity. Within the chamber the GWSS are grown in Bug Dorm insect cages each containing two cowpea, two cotton, and two basil plants in five-inch pots. The colony appears to be very robust with a full range of nymphal instars and adults. Oviposition of eggs occurs primarily on cotton and cowpea. Experiments to determine the development time of GWSS reared on basil are currently in progress. In these experiments, newly emerged nymphs are placed on a single basil plant (ca. 10 cm in height) that is placed in a cylindrical (10 x 24 cm) acrylic cage that is capped at one end with nylon mesh. These cages are also placed in the environmental growth chamber described above. The development of insects is scored daily. The current data indicate that the development times of each nymphal instar on basil (i.e., first instar: 5.3±0.5 days; second instar: 4.3±0.5 days; third instar: 5.0 days) are very similar to those found on sweet potato by Lauziere and Setamou (2009). In preliminary experiments in which first instar nymphs were exposed to the JHA methoprene, no mortality or delays in development (approximate 30 day long development time to adult eclosion) were found. These nymphs were exposed to the methoprene (0.2 ml of a 0.5 or 5.0 ppm solution applied to the surface of a 20- ml glass vial (32 cm^2 surface area)) for only one hour. As soon as we determine the exact development times of GWSS under our rearing conditions, a full complement of dose- and time-respnse experiments with methoprene and other JHAs will be performed.

II. Characterize authentic JH esterase (JHE) activity in GWSS

Experiments to characterize JHE activity in GWSS will be performed using precisely staged nymphs. Experiments to determine the developmental times of GWSS under our rearing conditions are still in progress (see Section I above).

III. Isolate *jhe* gene and characterize recombinant JHE protein

In order to clone potential JHE- and JHEH-encoding genes of GWSS, total RNAs were collected from 5th instar nymphs. The total RNAs (1.2 µg) were used for first strand cDNA synthesis using the poly-T primer CDS III/3' PCR (Clontech, Mountain View, CA). Attempts to PCR-amplify the 3'-end and 3'-UTR of the putative *jhe* and *jheh* genes of GWSS were made using numerous degenerate primers that recognized conserved sequences in known JHE and JHEH gene (reviewed in (Kamita and Hammock, 2010). Candidate amplicons following the PCRs are now being cloned into plasmid vectors and will be sequenced.

CONCLUSION

A little over two months have passed since we started this project, during this time we have established our laboratory colony of GWSS and are in the process of determining precise developmental times under our rearing conditions. Determination of precise developmental times is critical for quantifying the efficacy and effects of the JHAs, effects of JHE-inhibitors, and JHE/JHEH levels in GWSS. We expect to complete determining GWSS developmental times during the next month.

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