FINAL PROJECT REPORT
WTFRC Project Number: CP-11-100A and CP-11-100B

Project Title: Identification of neuropeptides and receptors in codling moth and SWD

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Other funding sources
None

Total Project Funding: $60,098

Budget History:

Item	**Year 1:**	**Year 2:**
Salaries	12,766.80	3,100
Benefits	1,688.51	949
Wages		
Benefits		
Equipment		
Supplies	13,593.69	16,000
Travel		
Plot Fees		
Miscellaneous **454 Sequencing**	12,000	
Total	40,049	20,049

ORIGINAL OBJECTIVES

1) Extract messenger RNA (mRNA) from heads of codling moth larvae, pupae and adults. Convert RNA transcripts to complementary DNA (cDNA). (Garczynski)

2) Determine sequences of cDNAs representing brain mRNA transcripts using 454 sequencing technology. (Dhingra)

3) Analyze sequences of assembled brain cDNAs to identify those encoding neuropeptides, peptide hormones, and other potential protein targets for codling moth control. (Dhingra, Garczynski)

4) Clone and characterize cDNAs from spotted winged Drosophila that encode neuropeptides and receptors involved in regulation of feeding and reproduction. (Garczynski)

SIGNIFICANT FINDINGS (ACCOMPLISHMENTS):

Year 1

- Gene transcripts encoding the spotted winged Drosophila sex peptide and its putative receptor have been cloned.
- Gene transcripts encoding spotted winged Drosophila neuropeptide F, short neuropeptide F, and their putative receptors have been cloned.
- A codling moth colony started from field collected insects was generated to provide insects more closely resembling those in the orchard. This colony was infused regularly with newly collected insects until sufficient numbers of insects were collected for RNA extraction and cDNA synthesis.
- Heads of thousands of codling moth larvae, pupae and adults were dissected and used to isolate mRNA for transcriptome sequencing and analysis.

Year 2

- Transcriptome of RNA extracted from codling moth larval, pupal and adult heads was completed
- A codling moth neuropeptide F receptor has been cloned and sequenced
- Codling moth neuropeptide F receptor was cloned into a mammalian expression vector and is currently being selected for expression in a mammalian cell line
- Expression of codling moth neuropeptide F has been detected in RNA extracted from adult male and female antennae

RESULTS AND DISCUSSION

Hormones are an organism's chemical messengers and a specific class, peptides (compounds consisting of two or more amino acid residues, the building blocks of proteins), regulate most every physiological function. Neuropeptides are peptides produced by cells in the brain and are released into the hemolymph (insect blood), sending signals to different tissues in the body. Because the hemolymph bathes virtually every cell in the insect body, circulating neuropeptides have the potential to come into contact with all tissues. Specific receptor sites form the connection between a circulating neuropeptide and particular target cell. When the neuropeptide interacts (binds) with its specific cell surface receptor, a signal is initiated causing the cell to perform a specific function. They work slowly, over time, and affect many different processes, including growth and development, metabolism, sexual function, and reproduction. Neuropeptides and hormones in general, are

powerful. It takes only a tiny amount to cause big changes in cells or tissues which is why too much or too little activity can cause serious affects. Because of critical functions of insect neuropeptides, they have been of interest for their use in pest control. Our overall goal was to generate a transcriptome from codling moth brain tissue, which is a known source of cells that express neuropeptides and neuropeptide receptors.

Cloning codling moth neuropeptide F receptor

The Neuropeptide F (NPF) family of peptide hormones regulates feeding and digestion in insects. For Drosophila, disruption of the NPF receptor (NPFR) inhibits larval food seeking and feeding behaviors. We used oligonucleotide primers designed from conserved amino acid sequences of known NPFRs in PCR reactions to clone a fragment of the gene transcript encoding codling moth NPFR. The nucleotide sequence of the NPFR fragment was determined and we then designed specific oligonucleotide primers that were used in PCR reactions to obtain the full length codling moth NPFR. The NPFR protein encoding region of the gene transcript is 1383 nucleotides in length and encodes a protein of 461 amino acids. The cDNA encoding NPFR has been cloned into a mammalian expression vector and has been used to transfect Chinese hamster ovary cells to generate a cell line that will be used in upcoming functional assays. These cell lines will be used in the future assays to discover compounds that may have antagonistic effects on receptor function perhaps leading to new codling moth control agents.

Codling moth neuropeptide F receptor is expressed in male and female antennae

Recently, it has been determined that a NPFR is expressed in Drosophila antennae, and it has been proposed that this receptor may signal the insect to express odorant receptors that have a role in detecting host or food seeking odorant cues. Based on these findings, we wanted to determine if codling moth NPFR is expressed in male and female antennae. Using oligonucleotide primers specific for codling moth NPFR, we performed PCR reactions with cDNAs prepared from RNA extracted from male and female antennae. In figure 1, we show that PCR amplifies products of the size expected for the codling moth NPFR protein encoding portion of its gene transcript (~1400 nucleotides). To confirm that this PCR product contains the NPFR protein encoding region, we cloned the PCR product and through sequence determination found it to indeed be a transcript encoding the codling moth NPFR.

Transcriptome generation and analysis

The “genomics revolution” has provided powerful new tools and information that might be used to discover novel protein targets for insect control. Our goal is to generate a transcriptome from codling moth heads, the site of neuropeptide and peptide hormone synthesis, in an effort to identify potential targets for the development of new compounds for codling moth control. RNA was extracted from heads dissected from all codling moth life stages and converted to cDNA for sequencing. The sequencing is now complete and the information is currently being analyzed. Analysis of the codling moth sequence information is not complete as of January 4, 2013 but will be continued until finished. As soon as we compile all the data and analysis is completed, we will send an addendum to Kathy Coffey so that she can forward it out to the Commissioners and committee members.

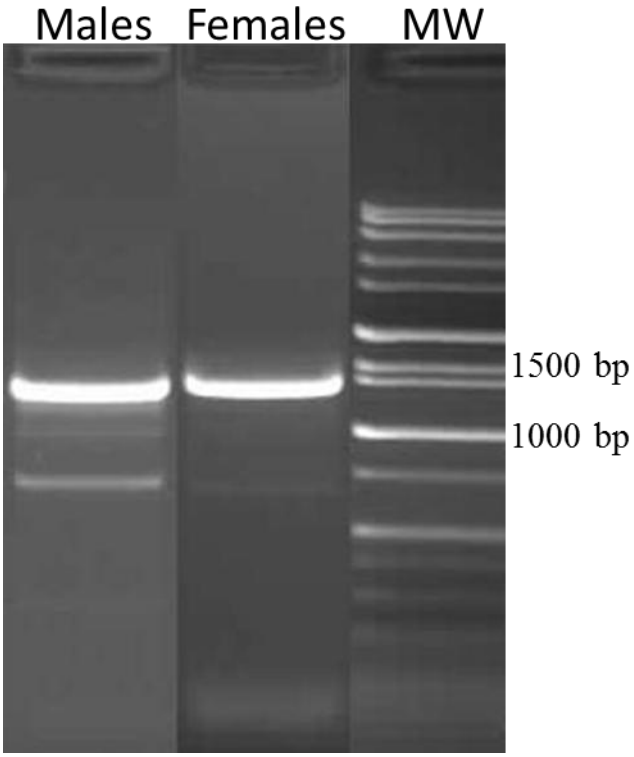

Figure 1. PCR amplification of codling moth NPFR from cDNA prepared from RNA extracted from male and female antennae. PCR was performed with primers specific for the codling moth NPFR protein encoding region of its gene transcript. The bright band at approximately 1500 base pairs in the male and female panels is the codling moth NPFR.

EXECUTIVE SUMMARY

Resistance to chemical insecticides used to control codling moth in the orchard is a major concern and is potentially costly to orchardists. Because many insecticides target proteins are produced by the brain and nervous system, the main goal of our project was to provide fundamental biological information on critical physiological functions of these organs through the generation and analysis of transcriptomes (a compilation of genes that are being actively expressed) from heads of various larval and adult stages of codling moth. Analysis of the transcriptome will be useful in identifying new protein targets that may be important in developing novel classes of compounds with unique modes of action that can be used in future resistance management programs to control codling moth. A second goal of this project was to initiate studies to identify cDNA transcripts encoding neuropeptides and receptors involved in the regulation of feeding and reproduction of spotted winged Drosophila (SWD), an emerging pest of tree fruit in the Pacific Northwest. This will provide basic information that can be used by other researchers in attempts to control this insect pest.

For SWD, we cloned gene transcripts encoding sex peptide and its putative receptor, as well as transcripts that encode for neuropeptide F, short neuropeptide F, and their putative receptors. These targets were chosen based on their roles in regulating reproduction (sex peptide) and feeding (neuropeptide F and short neuropeptide F). These clones have been made freely available to the research community in efforts to further characterize the neuropeptide/receptor interactions with the hope of developing control agents that disrupt their physiological function.

A codling moth neuropeptide F receptor was also identified and cloned as part of this project. Neuropeptide F regulates feeding and digestion and is expressed in codling moth neonates. Future work will be to fully characterize the interactions of this receptor with its neuropeptide ligand in attempts to exploit this system as a potential target for control of codling moth neonates. Interestingly, we detected the neuropeptide F transcript expressed in codling moth adult male and female antennae. Because neuropeptide F is involved in regulation of feeding, it is hypothesized that activation of this receptor in antennae turns on expression of gene transcripts encoding for receptors involved in host plant seeking or feeding behaviors. We will be examining this hypothesis in future studies addressing the codling moth olfactory system.

A transcriptome has been generated from RNA extracted from codling moth heads dissected from larvae and adults. The transcriptome annotations are being completed and we will be analyzing this data as it becomes available. It is anticipated that we will have a plethora of new information regarding proteins produced in the codling moth brain. This information will be used in the future to identify novel targets that may be used in the development of new compounds that can be useful in insect control efforts.