FINAL PROJECT REPORT

Project Title: Olfactory proteins as targets for enhanced codling moth control

WTFRC Project Number: CP-12-101

PI: Stephen F. Garczynski
Organization: USDA-ARS
Telephone: 509-454-6572
Email: steve.garczynski@ars.usda.gov
Address: 5230 Konnowac Pass Rd.
City/State/Zip: Wapato, WA 98951

Cooperators: Pete Landolt, Tom Unruh, Alan Knight (USDA, Wapato WA), Jocelyn Millar (University of California, Riverside), Walter Leal (University of California, Davis)

Other funding sources: *None*

Total Project Funding: $125,134

Budget History:

Item	2012	2013	2014
Salaries	$24,958	$25,714	$26,470
Benefits	$ 4,292	$ 4,333	$ 4,367
Wages			
Benefits			
Equipment			
Supplies	$10,000	$10,000	$10,000
Travel			
Plot Fees			
Miscellaneous	$ 5,000		
Total	$44,250	$40,047	$40,837

ORIGINAL PROJECT OBJECTIVES

1) Express and characterize proteins involved in codlemone detection. Proteins thought to be involved in the detection and regulation of pheromone (codlemone) signaling include pheromone binding proteins (PBP), sensory neuron membrane proteins (SNMP), pheromone receptors (PR) and odorant degrading enzymes (ODE). The purpose of this objective was to clone and produce material we could functionally analyze.

2) Determine which odorant binding proteins, nerve membrane receptors, and odorant degrading enzymes are involved in the codlemone signaling pathway using *in vitro* protein expression and binding assays. Using materials generated in objective 1, the goal of this second objective was to determine which proteins interact with codlemone.

3) Determine expression of olfactory protein gene transcripts and detect GOBP that are expressed in antennae using immunofluorescent detection methods. General odorant binding proteins (GOBP) and PBPs have been shown to bind pheromones in other moths. The goal of this objective was to generate antibodies that bind to GOBPs so that we would have a tool to detect these proteins in codling moth antennae. Once antibody detection was confirmed another goal of this objective was to determine if gene expression and protein production can be correlated.

4) Determine if codlemone signaling can be disrupted using various odorant degrading enzyme inhibitors and parapheromones in flight tunnel studies. Degradation of pheromones (codlemone) is thought to be important for maintaining the sensitivity of pheromone signaling and behavioral responses in male moths. The first goal of this objective was to use enzyme inhibitors to determine the class of ODE involved in degradation of codlemone. Parapheromones, molecules that are strong agonists or antagonists of pheromones (codlemone), have been developed for other moth species. The second goal of this objective was to determine if a parapheromone could be developed to disrupt codlemone activity in male moths.

SIGNIFICANT FINDINGS

Objective 1:
1) Identified five additional odorant binding proteins (OBP) including 2 PBPs (5 total) and 3 GOBPs (6 total), that have potential to serve as codlemone binding proteins.
2) Identified 2 additional SNMPs (4 total) that have the potential to function in codlemone signaling.
3) Identified 3 additional PRs (8 total).
4) Identified two additional ODEs (24 total).
5) Identified 25 chemosensory binding proteins (CSP). CSPs have been shown to bind pheromones in other moths.
6) Discovered a potentially new mechanism for regulating olfactory protein (odorant receptors including PRs, PBPs, GOBPs) production in antennae.

Objective 2:
1) Generated a new cell line to use in high-throughput assays for odorant receptor/ligand identification assays.

Objective 3:
1) Generated antibodies that can be used to detect GOBPs in antennae.
2) Quantitated PR gene expression using qPCR.

Objective 4:
1) Synthesized a parapheromone derivative of codlemone.
2) In a preliminary field trial determined that the parapheromone may be a codlemone antagonist.

RESULTS AND DISCUSSION

The overall goal of this project was to characterize olfactory proteins that interact with codlemone in an attempt to identify proteins that could be targeted for codling moth control. When we first started this project we had limited information with only a handful of proteins to evaluate. Through the WTFRC-funded codling moth transcriptome, we have substantially increased the number of proteins that need to be evaluated. We have now in total 54 transcripts encoding odorant receptors (8 belonging to the pheromone receptor sub-family), 48 transcripts encoding odorant binding proteins (5 pheromone binding proteins and 6 general odorant binding proteins), 25 chemosensory binding proteins, 24 odorant degrading enzymes and 4 sensory neuron membrane proteins. This result demonstrates the complexity of the codling moth olfactory system and is now providing more target proteins that need to be evaluated. Because the methods currently used for evaluation of each of these proteins are not set up to handle these numbers, new methods will be needed for thorough characterizations. To address this issue, I have initiated a collaboration with Dr. Sindhuja Sankaran, a professor in the Ag Engineering department at WSU, to develop a biosensor system that can be used as a high-throughput method to determine odorant ligands of the codling moth olfactory proteins that have been identified in this project. In addition, we are developing a gene knock out system (CRISPR/Cas9) that if successful, will provide us with a rapid method to evaluate olfactory protein function directly in the codling moth.

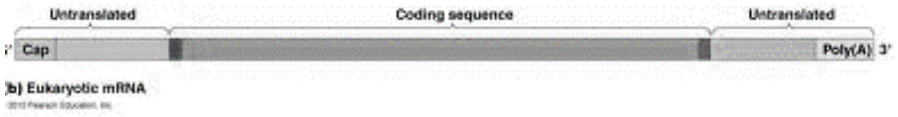

Figure 1. Diagram of messenger RNA structure. Messenger RNA (mRNA) has 5 structural features. The coding sequence is the portion of mRNA that is translated to produce the protein. The 5' and 3' ends of the mRNA are untranslated regions, but have features that protect mRNA from being degraded (Cap and Poly(A)). The untranslated regions are also involved in regulating localization and translation of the mRNA into protein.

An unforeseen result of this project, and perhaps the most exciting, was the discovery of a potential mechanism that insects use for the regulation of olfactory protein production. For years, researchers have been trying to use quantitative PCR (qPCR) as a method for determining which proteins are important in pheromone (and other odorant) detection. Using qPCR to examine olfactory protein production has had little or no success. We discovered that gene transcripts (messenger RNA; see Figure 1 for a diagram of mRNA structure) encoding olfactory proteins produced anomalous results. Because we are comprehensive in our analyses of PCR amplified gene transcripts, we found that multiple transcripts with identical coding regions but differ in the length of the 3' untranslated region of mRNA are produced for both odorant receptors and pheromone binding proteins. Why might this be important? Normally, when proteins are needed by cells, they generate an mRNA encoding that particular protein and the mRNA is translated into a protein right away for immediate use. It is now becoming clear, in mammals at least, that nerve cells (neurons) use a different mechanism for protein production. In this mechanism, mRNA is produced, transported to a region of the nerve cell where the protein is needed and then the mRNA is locally translated when the protein is needed. Because of this mechanism, quantification of mRNA does not correlate with protein production, explaining why qPCR is not a viable method for evaluating olfactory proteins. Therefore,

alternative methods will be needed in the future to evaluate protein production in response to pheromones. We are currently developing a method using 2-dimensional gel electrophoresis to evaluate proteins that are produced in response to codlemone exposure. Because this method will be using techniques and tools developed in Objective 3 of this project, I hope to provide you with further information in the future.

EXECUTIVE SUMMARY

The use of codlemone for mating disruption has had major impact in codling moth control programs. The major sensors that regulate codlemone detection and behavior are proteins that reside in the olfactory neurons located in codling moth antennae. With a greater understanding of how this detection system functions, new compounds or methods might become apparent for enhanced control of codling moth through disruption of olfactory proteins. Therefore, the main goal of this project was to identify and characterize proteins that participate in the detection and regulation of codlemone.

In previous WTFRC-funded projects, we used a PCR-based method to identify five putative pheromone receptors, and most recently, we mined a codling moth transcriptome which led to identification of three additional receptors. Through quantitative PCR analyses of transcripts expressed in male and female antennae, one transcript was determined to have male biased expression at extremely high levels. The transcript encoding this receptor was cloned and expressed in a mammalian cell line to determine if it is a codlemone receptor. In cell-based assays, addition of codlemone elicited a cellular response indicating it is a codlemone receptor. However, we do not know if it is the codlemone receptor used by codling moth males to locate female mates. We are working to develop a genome editing system where we can knock out our putative codlemone receptor and then use flight tunnel bioassays to determine receptor function in behavior response. In addition to the pheromone receptors, the codling moth transcriptome has been mined to identify 54 transcripts encoding odorant receptors, 48 transcripts encoding odorant binding proteins, 22 transcripts encoding odorant degrading enzymes, 25 transcripts encoding chemosensory binding proteins and 4 transcripts encoding sensory neuron membrane proteins. In the future, projects to determine the roles of these proteins in codling moth will be needed to gain a fuller understanding of olfaction mechanisms.

An interesting finding in this project was that a high proportion of transcripts encoding olfactory proteins contain modified 3' untranslated regions. Two mechanisms we found that cause these modified 3' untranslated regions are alternate polyadenylation and differential splicing. In both of these mechanisms, nucleotide sequence is deleted, perhaps modifying response elements present in the 3' untranslated regions that regulate transcript localization or have effects on translational control. In mammals, similar mechanisms are used in nerve cells to regulate mRNA localization and translation of nerve proteins. We will be exploring the significance of modified 3' untranslated regions in codling moth olfactory protein transcripts to determine the relevance of this observation in regulation of protein production in olfactory neurons.

The results produced from this project have generated several other ideas for future research. We produced a codlemone analog that in preliminary field trials appears to act as a codling moth deterrent. We will continue pursuing this line of research to determine if there are field applications for this compound in codling moth control efforts.