AGRICULTURAL RESEARCH FOUNDATION
FINAL REPORT
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TITLE:

Development of a mealybug-detecting biosensor for the protection of Oregon's wine industry

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SUMMARY/ABSTRACT:

Grapevine leafroll disease (GLD) is one of the most destructive and economically devastating diseases of the Oregon grape industry. This disease is caused by a group of viruses that are transmitted to healthy vines by several species of mealybugs. There currently is no cure or treatment for GLD, and the only way to prevent its spread is to remove virus-infected vines while simultaneously controlling mealybug populations. A biosensor capable of detecting mealybugs in the field would have various applications that would benefit Oregon's viticulture industry. In addition to improving our ability to detect and monitor mealybug populations in vineyards, these sensors could be spread over larger geographical regions and linked to automated reporting systems that could monitor the spread of these pests throughout a region. Individual growers would know exactly how exposed they were to the risk of GLD spreading over time if they had infected vines, or detect a mealybug infestation early enough to prevent significant losses from transmission of GLD. For growers with established mealybug populations, the sensors could be used to localize insecticide treatment programs to infested vines, making spray programs more efficient and effective, which would directly benefit the environment through reduced use and off-target effects.

The aim of this research project was to catalyze the development of an electronic nose designed to localize mealybugs in vineyards by identifying the proteins involved in pheromone reception in mealybugs. Towards this end, we generated genetic resources from two mealybug species and used a bioinformatic approach to identify proteins with putative roles in pheromone detection in these species. We then produced recombinant versions of some of these candidate proteins and validated their role in pheromone detection in functional studies. While this research project was heavily impacted by the Covid pandemic, significant progress was still made, in that a pheromone receptor for one of these species was identified, which will support further development of a mealybug-detecting biosensor.

OBJECTIVES:

We aimed to identify the proteins involved in pheromone reception in the grape mealybug, *Pseudococcus maritimus*, and the vine mealybug, *Planococcus ficus*, two of the most economically damaging mealybug species to the grape industry. The specific research goals of this ARF proposal were:

1) To generate genomic and transcriptomic libraries from *P. maritimus* and *P. ficus*
2) To identify candidate binding proteins, pheromone receptors and degrading enzymes through phylogenetic and expression analyses of these genetic libraries
3) To validate the function of candidate pheromone-related proteins through expression and testing in existing assay systems

PROCEDURES:

Objective 1: Generation of genomic and transcriptomic libraries from *P. maritimus* and *P. ficus*.

Vine mealybugs were collected from an established colony maintained by Kent Daane at the Kearney Agricultural Research Center in Parlier, CA. Grape mealybugs were collected from infested vineyards in Boone County, Missouri. The genetic identities of specimens were validated by DNA barcoding prior to generation of genomic and transcriptomic libraries via Illumina Next-Generation sequencing technology by a commercial third party (Novogene). Draft genomes were produced for both species, three replicate transcriptomic libraries were produced from both male and female vine mealybugs, and three replicate transcriptomic libraries were produced from female grape mealybugs.

Objective 2: Identification of candidate binding proteins, pheromone receptors and degrading enzymes through phylogenetic and expression analyses of genetic libraries.

The DNA sequences encoding vine and grape mealybug olfactory proteins were identified from genomic and transcriptomic libraries by BLAST analyses using the sequences of known olfactory proteins from other insects as queries. In total, 52 putative odorant receptors, 18 putative odorant binding proteins and 14 putative odorant degrading enzymes were identified from the vine mealybug genetic databases. Eight odorant receptors were found to be highly expressed in the male transcriptome and were chosen for expression and testing in cell-based assays. In total, 42 putative odorant receptors, 10 putative odorant binding proteins and 17 putative odorant degrading enzymes were identified from the grape mealybug genetic databases.

Objective 3: Validation of candidate pheromone-related protein function through expression and testing in existing assay systems.

Candidate vine mealybug sex pheromone receptors identified from genomic and transcriptomic analyses were cloned from RNA, ligated into plasmid vectors and expressed in HEK293 cells. Cells stably expressing each receptor, along with the obligate olfactory receptor co-receptor (Orco), were tested for responsiveness to the vine mealybug sex pheromone, lavandulyl senecioate. Of the eight receptors tested in cell-based assays, only one receptor responded to the vine mealybug sex pheromone in a dose-dependent manner. Unfortunately, due to restrictions enacted in response to the Covid pandemic, it was not possible to test candidate grape mealybug pheromone receptors in cell-based assays during the duration of this project.

SIGNIFICANT ACCOMPLISHMENTS:

- Sufficient quantities of RNA and DNA were collected from vine and grape mealybugs to facilitate genetic analyses
- Draft genomes were produced for both mealybug species
- Transcriptomic libraries were produced from male and female vine mealybugs
- Transcriptomic libraries were produced from female grape mealybugs
- Members of the odorant receptor, binding protein and degrading enzyme gene families were identified and annotated in the genomes, and open reading frames of olfactory genes were confirmed and gene expression was analyzed via transcriptomic analyses
- Eight receptors were found to display high, male-biased expression in vine mealybug
- A receptor for the vine mealybug sex pheromone was identified through functional testing in cell-based assays

BENEFITS & IMPACT:

This research project facilitated the identification of olfactory gene family members in two economically relevant mealybug species. These gene family members will likely serve as targets in next-generation insecticide development programs in future studies. The vine mealybug sex pheromone receptor identified in this research project will facilitate the attempted development of a mealybug-detecting biosensor in future research projects.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

None

FUTURE FUNDING POSSIBILITIES:

This research project produced the preliminary data required to advance this project towards the next step, the development of a mealybug-detecting biosensor. The data generated from this OSU Agricultural Research Foundation grant will be used to support a multi-institutional application for funding from the USDA – NIFA or USDA – AFRI.