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NATIONAL PEANUT BOARD/SOUTHEAST PEANUT RESEARCH INITIATIVE
QUARTERLY PROGRESS REPORT FOR WORK DONE UNDER RESEARCH AGREEMENT

Report: Final report

INSTITUTION: University of Georgia

Project Title: Examining issues associated with the efficacy of two neonicotinoid insecticides (CruiserMaxx® and Admire Pro®) against thrips on peanut in comparison with Thimet®

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Summary

Imidacloprid and other neonicotinoids such as thiamethoxam have been considered as viable alternatives to widely used broad-spectrum insecticide, Phorate. We have extensively worked in the past to integrate their usage into peanut production, and have published exclusively on their usage in peanut production (Marasigan et al. 2016 and 2018). Their efficacy seems to be sufficient to suppress early season thrips injury and suppress virus transmission in comparison with phorate. However, research reports in 2015 and 2016 have highlighted the widespread resistance in thrips against neonicotinoids in cotton. We evaluated these compounds for thrips susceptibility routinely from 2016 to 2018. Results thus far indicated that thrips resistance to neonicotinoids does not seem to be prevalent in peanut in GA. We have also been working towards developing resistance quantitation assays for detoxifying enzymes using transcriptome mining. This approach will aid to identify resistance quickly. This research is ongoing; faced with poor resolution, the goal is now geared towards developing a thrips genome to increase our resolution in development of these assays.

This work is a multi-year continuation project. The work so far has examined residual toxicity of nenonicotinoid insecticides and thimet in peanut foliar tissue and correlated that to thrips mortality over time. We have been regularly comparing Lc50 values of lab populations to field populations. Thrips populations were collected from fields in at least 10 counties routinely in 2016 and 2017 and assayed for reduction in susceptibility to imidacloprid. The susceptibility of these insecticides in field insects was compared with our lab colony, which is being maintained since 2009. Screening has not provided substantial evidence for increased resistance in selected field populations in Georgia. The resistance ratio i.e., Lc50 of field thrips/Lc50 of lab thrips has largely remained close to 1 indicating the absence of thrips resistance to imidacloprid and/or

thiamethoxam in peanut. This information is critical, as there is a major shift among peanut producers to transition from using phorate to imidacloprid. It is estimated that approximately 40% of the peanut acreage is under imidacloprid usage.

While these bioassays with live thrips often provide a good measure of thrips susceptibility to imidacloprid, it often requires collection of live insects, transport, maintenance of a lab colony, and time consuming bioassays. To improve our resistance detection efficiency, we are hoping to develop rapid detoxification enzyme quantitation assays using qPCR.

Transcriptome mining: with funding from NPB we have been developing transcriptomes for *F. fusca* (thrips), this effort was undertaken to assess how TSWV affects thrips. These transcriptomes have already been developed, and results have already been published (Shrestha et al. 2017).

Detoxifying enzymes in thrips such as Cytochrome P450 have been implicated in the resistance against neonicotinoids, especially against imidacloprid. Thrips are 'R' strategists capable of exploiting a wide range of host plants. They have evolved strategies to combat xenobiotics that are plant derived, this successful evolution background against xenobiotics has primed thrips to use the same/similarly-acquired strategies to combat insecticides. One of the weapons in their arsenal is the P450 enzyme detoxification system. Research conducted since the 1980s has clearly documented the role of P450 enzymes in detoxification of multiple insecticide classes such as organophosphates, carbamates, pyrethroids, and neonicotinoids.

The genome for *F. fusca* has not been sequenced. Nevertheless, our laboratory has been involved in developing transcriptomes and examining differential gene expression following the acquisition of a propagative virus, TSWV. The adult *F. fusca* transcriptome is available through the NCBI database (Accession no. SRX286866). Transcriptomes for varying life stages were synthesized *de novo*. This allowed us to specifically mine the transcriptomes for P450 genes in *F. fusca*. This was accomplished first by developing a database with known nucleotide sequences of P450 genes from several hexapods including *Aedes ageypti, Anopheles* and *Culex* sp., *Bemisia tabaci*, *Drosophila melanogaster*, *Dendroctonus armandi*, *Frankliniella occidentalis*, *Myzus persicae*, *Plutella xylostella*, and *Tribolium castaneum*. The sequences were blasted against the *F. fusca* transcriptome. Subsequently, 31 *F. fusca*-specific P450 sequences that showed substantial homology to the query sequences in the database were identified with a stringent E value of at least $1E^{-4}$ or less. The percent identity and length of the alignment were also taken into consideration. A sample table below indicates some of the blast output parameters that were used to choose sequences from the *F. fusca* transcriptome.

Table 1. Sequences in F. fusca transcriptome homologous to P450 sequences in other insects

Query	Subject	%id	Alignment length	E value	Bit score
comp70824_c0_seq1	*Thrips palmi* CYP6	29.79	235	1.00E-21	85.1
comp58661_c0_seq5	*Drosphila melanogaster* Cyp6g1	20.85	753	1.00E-14	71.6
comp56481_c1_seq10	*Dendorctonus armandi* CYP18A1	25	348	1.00E-14	72.4
comp57772_c0_seq3	*Dendorctonus armandi* CYP18A1	25.14	366	1.00E-15	76.3
comp52368_c0_seq1	*Frankliniella occidentalis*CYP6EC1	25.49	514	1.00E-14	72.8
comp48237_c0_seq1	*Plutella xylostella* CYP9G2	29.75	158	1.00E-11	63.5
comp32616_c0_seq1	*Frankliniella occidentalis* CYP6EC1	23.59	462	1.00E-12	66.2
comp59396_c0_seq1	*Dendorctonus armandi* CYP18A1	26.15	348	1.00E-17	83.2
comp59394_c4_seq1	*Frankliniella occidentalis*CYP6EC1	22.97	505	1.00E-12	67
comp59784_c0_seq12	*Dendorctonus armandi* CYP18A1	24.14	1823	1.00E-58	217
comp41984_c0_seq1	*Frankliniella occidentalis*CYP6EC1	25.57	352	1.00E-13	70.1
comp58095_c0_seq1	*Drosphila melanogaster* cyp6a2	25.45	727	1.00E-29	123

The identified sequences were then examined for evolutionary relationships using Bayesian Markov Chain Monte Carlo (MCMC) analysis in Mr Bayes. A representative phylogenetic tree is included below (Fig. 1). The analysis revealed that at least 14 P450 sequences were in clades with other characterized P450 gene sequences. *F. fusca* transcriptome sequences fell in the same clades as that of P450 sequences belonging to *Aedes aegypti*, *Bemisia tabaci*, *Drosphila melanogaster*, *Dendroctonus armandi*, *Frankliniella occidentalis*, *Plutella xylostella*, *Thrips palmi*, and *Tribolium castaneum*. These sequences are all highlighted in green. Thus it is possible that some of these genes could be involved in similar detoxification roles as that of their clade members. Despite these similarities, functional/biological characterization is necessary to

confirm their role in neonicotinoid detoxification. The homologous *F. fusca* sequences were similar to clade members from four CYP families viz., CYP1, CYP3, CYP4, and CYP4. Genes in CYP4 and 6 are closely associated with pesticide detoxification in *F. occidentalis*.

Also, by following the same procedure described above for P450, we were also able to identify homologous sequences of carboxyl esterase, super oxidase, transferase, acetylcholine esterase, and acetylcholine esterase receptor in *F. fusca* transcriptome (data not presented here).

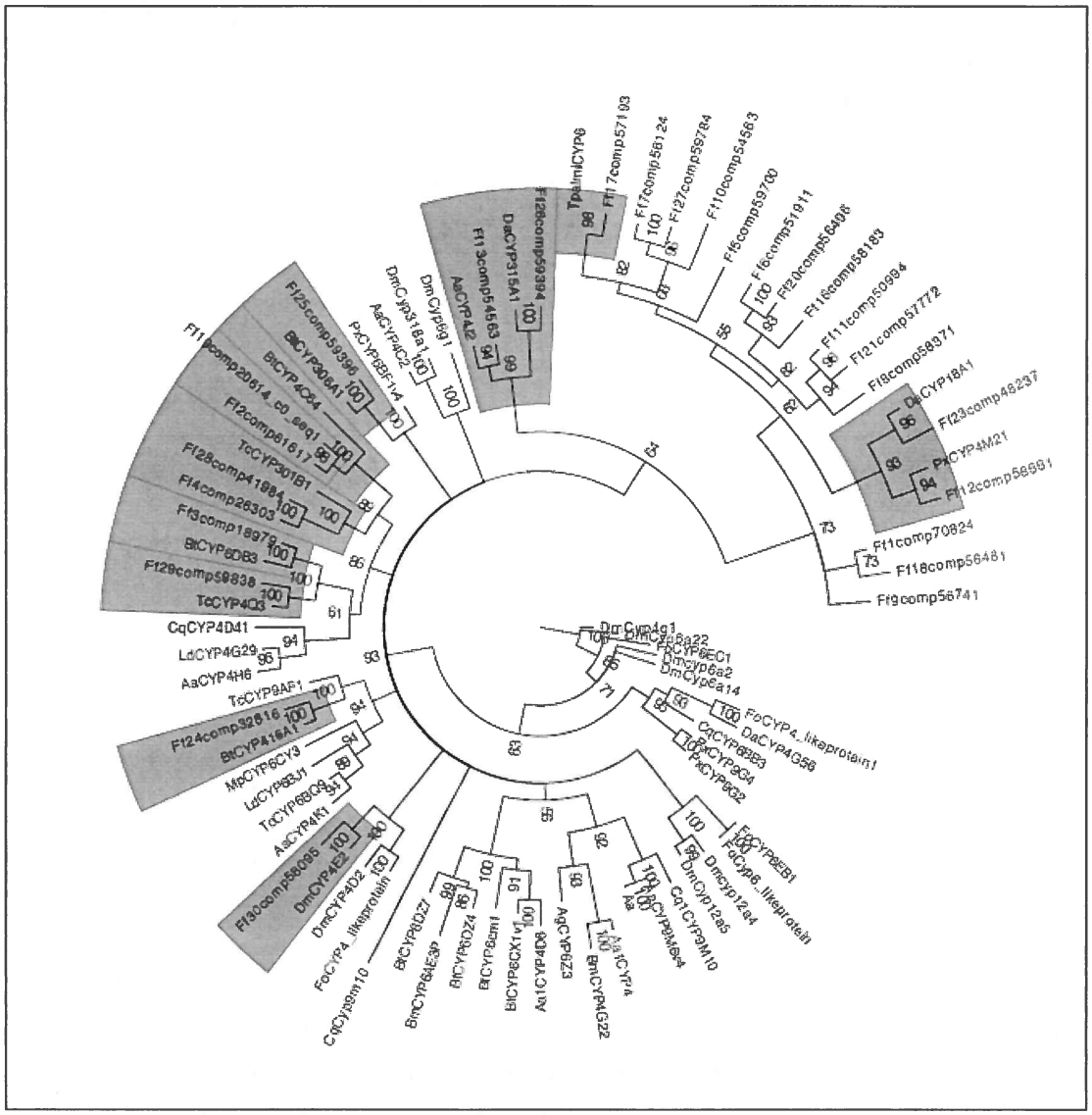

Fig. 1. A cladogram depicting relationships between P450 gene sequences obtained from F. fusca with other insects.

As a first step, primers were synthesized for several homologous sequences representative of P450 genes in *F. fusca* using the NCBI primer-designing tool Primer-BLAST

http://www.ncbi.nlm.nih.gov/tools/primer-blast/. The primers were then subsequently used to detect P450 associated genes in *F. fusca* samples using standard Polymerase Chain Reaction (PCR) protocols. Preliminary results indicated that several putative P450 genes were detectable, and that PCR and qPCR-based detection and quantification of P450 genes from thrips samples should be possible. The figure below shows P450 specific bands in 1% agarose gel containing cDNA samples of *F. fusca*. PCR product purification and Sanger sequencing further confirmed the specificity of the bands.

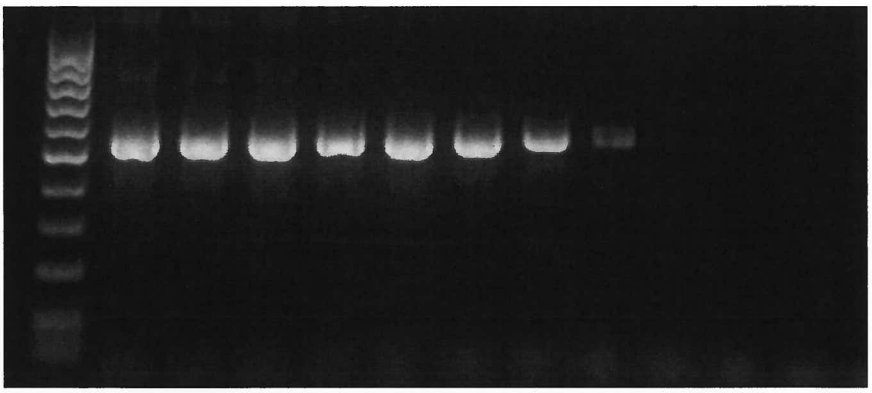

Fig 2. An agarose gel photograph showing ~500 bp representing P450 gene products on a temperature gradient ranging from 55 to 61.7 ℃.

However, with so many homolgous sequences present and low sequence homology with data base members, and with no resistant colony present, this work is not ready for prime time yet. The lack of specificity of these sequences could be very challenging to design assays, especially when our targets belong to multigene families as in the case of P450. With advancements in technology, it would be better to address this issue through the genome route. We will be developing a *F. fusca* genome shortly, and work is currently in progress to achieve the same. Sequencing is anticipated to be completed before the end of the year, and annotation will proceed later. We hope to obtain better quality detoxification enzyme sequences based on the draft genome assembly. This work has been of interest to folks in Alabama (Auburn) and in North Carolina (NCSU). Collaborations are in effect to attain this research goal. We anticipate development of such assays and validate their usefulness by the end of 2019.

References

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