AMCARF Project Status Report

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Report Type:

	Report Type	**Report Deadline**
	Progress Report	July 15th, 2020
√	Annual Report	December 14th, 2020

Project Title: Non-target effects of autodissemination approaches for mosquito control

AMCARF project number: 2020-02

Project Cost: $24,240.40

Project Leader: Corey Brelsfoard, Ph.D.

Collaborators: (Including cooperating laboratories and AMCARF supported personnel and percent effort).

Scott Longing, Ph.D. (TTU) (10% Annual effort)

Project Objectives:

Objective 1. Nectar source and pollinator cross contamination in laboratory cages. To examine for contamination of nectar sources and pollinators with PPF (pyriproxyfen) via the ADAM (autodissemination augmented by males) approach, we will setup replicate cages with PPF dusted *Ae. albopictus* males, conspecific *Ae. albopictus* females, artificial nectar sources, oviposition cups, and *A. mellifera*. Here we will assess the potential for PPF dusted male mosquitoes to contaminate artificial nectar sources and cross contaminate *A. mellifera* to validate the risks of non-target effects of the ADAM approach.

Objective 2. Non-target effects of autodissemination approaches in semi-field cages. Experiments will assess the potential for non-target effects and contamination of nectar sources and pollinators in a semi-field cage setting. Similar to objective 1, we will assay for the presence of PPF on pollinators and natural and artificial nectar sources placed in field cages from PPF dusted males. Secondly, we will examine the potential for *Ae. albopictus* to deliver PPF to nectar sources and to cross contaminate pollinators by visiting autodissemination stations and returning to nectar sources.

Total Project Progress:

The Brelsfoard lab was granted permission to return to work with a limited work schedule as of June 10th 2020 due to the Covid-19 pandemic. However, with a limited work schedule we have made considerable research progress related to objective 1 and are working towards completion of objective 2 with some minor modifications due to timeline changes and the lack of the ability to run field cages in the winter. We are currently preparing a manuscript describing the laboratory experiments outlined in Objective 1. Key research accomplishments are outlined below.

Key Research Accomplishments:

Objective 1 accomplishments: Nectar source and pollinator cross contamination in laboratory cages.

Brief methods:

- To examine for non-specific transfer of pyriproxyfen (PPF) from ADAM males to artificial nectar sources and *Apis mellifera* workers, we setup four cage types containing dusted or undusted *Ae. albopictus* males, *Ae. albopictus* females, and *A. mellifera* workers, which are outlined in Figure 1. All cage types were replicated four times. Male and female mosquitoes, and bee mortality was recorded every 24 hours. After five days, the trial was terminated by closing each cage type. Water from oviposition cup, filter paper, cotton wick, honeybees, male and female mosquitoes were collected and stored at -20 °C until for later use in bioassays and for mass spectrometry analysis (LC-MS). All insects and materials were washed in 1 ml of methanol (HPLC grade) and the supernatant used for LC-MS (Figure 1).

Results:

- *PPF transfer demonstrated via fluorescent imaging.* We were able to successfully show that PPF is indirectly transferred to artificial nectar sources and *A. mellifera* using a PPF and fluorescent powder mixture to dust *A. albopictus* males. Images of artificial nectar source materials collected from cage type one show there is transfer of PPF directly and indirectly from PPF treated *Ae. albopictus* males and *A. mellifera*, respectively to the cotton wick (Figure 2 A, B and C, D) and filter paper ring surrounding the cotton wick on the artificial nectar sources (Figure 2 E, F and G, H). PPF was directly observed on dusted *Ae. albopictus* males when compared to undusted males (Figure 3 A, B and C, D). Indirect PPF transfer was also observed to *A. mellifera* in cages housing PPF dusted *Ae. albopictus* males (Figure 3 E, F and G, H). PPF direct and indirect transfer to *Ae. albopictus* females was also observed shown by the presence of fluorescent powder on untreated females placed in cage type 3 (Figure 3 I, J and K, L). No evidence of PPF transfer was observed in cage type four containing untreated *Ae. albopictus* males (Figure 3 C and D).

- *Bioassays.* To examine for dissemination of PPF from treated males directly to nectar sources and oviposition containers, and indirectly to female *Ae. albopictus* and *A. mellifera* we performed a series of bioassays on insects, oviposition cup water, and artificial nectar source materials from four cage types with a different combination of PPF dusted and undusted *Ae. albopictus* and *A. mellifera* (Figure 4). A significant lethal effect was observed in bioassays containing materials from an artificial nectar source, *A. mellifera*, and PPF treated *Ae. albopictus* males collected from cage type one containing PPF treated males and *A. mellifera* (ANOVA, F =

34.4, DF = 6, P <0.0001) (Figure 4). To examine for the dissemination of PPF from treated males to nectar sources and oviposition containers without the presence of *A. mellifera*, cage type two consisted of only PPF treated *Ae. albopictus* males and untreated females (Figure 1). A significant lethal effect was observed in bioassays containing *Ae. aegypti* PPF treated males and materials from the artificial nectar source collected from cage type two (ANOVA, F = 22.0, DF = 6, P < 0.0001) (Figure 4). To examine for direct and indirect PPF transfer, cage type 3 consisted of PFF untreated males, untreated females, *A. mellifera*, and an artificial nectar source. A significant lethal effect was observed in bioassays containing *A. mellifera*, PPF treated males, and materials from the artificial nectar source (ANOVA, F = 70.0, DF =7, P < 0.0001) (Figure 4). Lastly cage type 4 consisted of untreated *Ae. albopictus* males and females, *A. mellifera*, and an artificial nectar source. No significant lethal effect was observed in bioassays containing untreated male and female *Ae. albopictus*, the cotton wick, and the filter paper surrounding the wick. However, a low level of mortality was observed in *A. mellifera* in bioassays and a high level of mortality was observed in bioassays directly inoculated with PPF (ANOVA, F =24.9, DF = 7, P < 0.0001) (Figure 4).

- *Survivorship assays.* Survivorship analyses were performed to examine for an effect of PPF on dusted *Ae. albopictus*, and females and *A. mellifera* exposed to indirect transfer of PPF. No difference in male *Ae. albopictus* survivorship was observed when comparing PPF treated to untreated individuals in the four cage types (Log-rank, Chi-square = 2.42, DF = 3, P = 0.49) (Figure 4B). Greater than 70% of females were observed to be alive on day five when cages were closed. A difference was observed in the survivorship of females in comparisons of females in cage types two, three, and four (Log-rank, Chi-square = 7.87, DF = 3, P = 0.20), wherein 88% of females in Cage type two were observed to be alive on day five compared to 76% and 73% of females in Cage type three and four respectively (Figure 4C). No difference in bee survivorship was observed in cage types one, three, and four (Log-rank, Chi-square = 0.59, DF = 2, P = 0.75). Bee mortality was ~25-30% in all cage types at day five (Figure 4D).

- *Mass spectrometry.* To examine for PPF on insects and indirect transfer to artificial nectar sources we performed liquid-chromatography-mass spectrometry (LC-MS) analyses on collected materials. Results suggest the presence of PPF on *A. mellifera*, *Ae. albopictus* females, and artificial nectar source materials from cages where PPF dusted males were released (Figure 5). The presence of PPF on the artificial nectar source and *A. mellifera*, again suggested PPF dusted males can contaminate nectar sources where important potential insect pollinators visit and indirectly contaminate *A. mellifera*. Additional, samples from cage types 1-4 are being examined for PPF using LC-MS. We expect to receive the results by early January 2021.

Objective 2 accomplishments: Non-target effects of cross-contamination of autodissemination approaches in semi-field trials

Brief methods:

- To assess non-target approach in a semi-field trial, we released *Ae. albopictus* males dusted with PPF into semi-field cages with conspecific females, *A. mellifera* foragers, and natural and artificial nectar sources. Treatments included PPF dusted males with *A. mellifera* to examine for indirect PPF to pollinators and without *A. mellifera* to examine for direct dissemination of PPF to nectar sources without the presence of pollinators. Control cages included *Ae. albopictus*

undusted males and females, and *A. mellifera*. We also plan to examine non-specific transfer from autodissemination traps in a similar experiment as described above.

Results:

- To complete this objective, we have setup multiple 12 x 12 hexagon metal frame field cages to test for the survival and foraging behavior of honey bees at the Quaker farm at Texas Tech University. However, initial attempts to release honey bees in field cages have been met with difficulties. While *A. mellifera* were observed to forage, their survivorship/longevity was less <24 hrs. Unfortunately, field cages were setup later than expected, and temperatures were extreme in West Texas reaching >110° F in mid-July, which affected *A. mellifera* and *Ae. albopictus* survivorship and *A. mellifera* foraging behavior. Additional, plants were grown in multiple locations in addition to the TTU biological sciences greenhouse for additional field tent trials later this summer/fall (i.e., September-October). We were able to complete two replicate experiment cages examining for transfer of PPF from dusted males in a semi-field setting in October (Figure 6). Insect and plant material from the cages were collected for analysis via bioassays and mass spec. We were also able setup a tent cage with an autodissemination trap and observed female *Ae. albopictus* entering and exiting the trap (Figure 2). Gravid females were tested for the presence of PPF after 24 hrs in the cages using bioassays, and all females tested positive for the presence of PPF (10/10).

Figure 6. Semi-field cage setup at the Quaker farm at Texas Tech University.

- To continue to make progress on this objective during the winter months we are currently performing tent cage studies inside of the Texas Tech Department of Plant and Soil Sciences greenhouse. In addition, these cage studies will examine for indirect transfer of PPF to additional pollinators other than *A. mellifera*. Specifically, we are examining for indirect transfer to Painted Lady Butterflies. We are confident we can address hypotheses in Objective two with the greenhouse cage studies this winter and additional semi-field cage studies in early spring.

<u>Reportable Outcomes:</u>

Describe major outputs including for example papers, inventions filed and patents issued, or new mosquito control guidance or practices.

We do not have any reportable outcomes at this time.

Results:

Progress Assessment:

Objective 1. We have successfully developed protocols to obtain honey bees from local colonies and demonstrate they will forage on artificial nectar sources in laboratory cages (100% completed).

Objective 1. Four replicate experiment examining laboratory cages for non-specific transfer of pyriproxyfen have been completed. PPF treatments consisted of a 1:1 mixture of fluorescent powder and Esteem 35 WP (35% pyriproxyfen). Examination of bees under a UV light shows cross contamination of PPF to honey bees collected from cages with PPF dusted male *Ae. albopictus*. Bioassays suggest non-specific transfer of PPF to artificial nectar sources, *A. mellifera*, and *Ae. albopictus* females. Insect and nectar source materials have been collected and examined for PPF transfer via bioassays (100% completed).

Objective 1. Mass spec. material preparation and PPF extractions, and mass spec. analyses from lab cage studies. We are waiting on the analysis one additional set of samples at the TTU Chemistry Department (80% completed).

Objective 2. Autodissemination stations have been constructed and tested for field cage experiments. *Ae. albopictus* males and females readily enter traps and become contaminated with PPF. (100% completed).

Objective 2. *A. mellifera* survivorship in field cages was observed to be < 24 hrs. Due to high summer temperatures, field cage experiments to examine for non-specific transfer of PPF to *A. mellifera* and flowering plants from the PPF treated males and autodissemination traps was delayed. Two replications of semi-field cages were completed in October/November and plant samples are awaiting analysis via bioassays and mass spec. Because of the change in timing of field cages due to weather and the Covid-19 pandemic, we are utilizing large tent cages in a greenhouse setting for additional testing over the winter months. We are also currently rearing different pollinator insects that may have a higher survivorship in field cages to use for experiments (i.e., Painted Lady Butterflies). Additional field cages will also resume in the Spring (25% completed).

Objective 2. Mass spec. material preparation and PPF extractions, and mass spec. analyses from semi-field and greenhouse cage studies (0% completed).

Green = on or ahead of schedule or successfully completed

Amber = slight delay but will meet all deliverables 1-6 months (specify) late

Red = major obstacles with a delay of more than 6 months, risk that key portions of the project will not be completed

Black = project was abandoned.

Plans for the following year: We expect to complete all laboratory experiments and a some of the field cage experiments by early spring 2021, and will be able to present available experimental results at the AMCA virtual annual meeting March 1-5. We have one manuscript almost completed describing the laboratory experiments, which we plan to submit to PLos NTD. An additional manuscript will be prepared describing the semi-field cage experiments examining for non-specific transfer of PPF to nectar sources and pollinator insects.

Conclusion: Despite the delays due to the Covid-19 pandemic, research progress as has been better than expected. At the conclusion of the project, we will have demonstrated the ability of PPF contaminated *Ae. albopictus* males, whether that is from a direct dust application or via an autodissemination trap to contaminate nectar sources and indirectly contaminate insect pollinators. The results will be discussed in reference to the potential nontarget effects of autodissemination approaches. We are grateful for the no-cost extension for this proposal to complete this important work.

IMAGES REDACTED FOR WEB (PUBLIC) VERSION

Images submitted in final report were submitted for publication to a journal.

When the publication when the is publicly available a link will be provided.