Final report 2018:
Title of project: Determining biocontrol options for peanut pests: a molecular approach

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Summary
Current control methods for the peanut burrower bug, *Pangaeus bilineatus* (Say), primarily based on insecticides have been unsuccessful. In addition, the over use of insecticides in attempts to control this pest can cause serious problems with other pests. These results suggest that biological control is occurring for secondary pests, and preserving these species is important. Our goal is to determine which of the predators in peanut systems are contributing to biological control on caterpillar pests, thrips, and determine if there are predators actually feeding on the burrower bug. We successfully designed a burrower bug primer that can be used for further analyses of predation on this pest, and a number of predators were observed positive for recent feeding on burrower bug. Our initial results screening over 2,000 predator samples provide evidence that predator food webs in peanut crops are complex. And, there are many predators feeding on aphids, thrips, occasionally on stink bugs, and alternative non-pest prey such as collembolan (a mostly soil dwelling insect) and flies are sustaining predator populations. We also successfully determined that this molecular approaches can be used to investigate fire ant roles in peanut systems. From our initial data set of 376 ant samples for a total of over 1,128 individual ants, these ants are eating on aphids, flies, and caterpillars occasionally. Further analysis would reveal the full extent of predation by ants and what aspects of the environment are favoring the population growth of ants in peanut crops.

Results and accomplishments of research objectives targeted for 2018:

1) Develop system to test for predation on burrower bug

Using methods employed in the Schmidt lab in the past and consistent with common practice, we designed a molecular tool to test for the presence of burrower bugs in predators. This allows for testing if predators found in fields had recently fed on burrower bugs, and if so, which predators provide any biological control for this pest. To create the molecular system, we first had to determine DNA sequences, primer probe for burrower bug, that were unique to the burrower bug, and test these for specificity, and for what conditions let to the best results for determining predation. We conducted a gradient temperature test with related species of burrower bug, species in the family, Cydnidae, and unrelated insect groups that we would expect to find in our samples (Figure 1). The results from this show that the burrower bug probe is 100% for only detecting burrower bug in a sample, and the best temperature was 56.7ºC (Figure. 1). We then combined this probe with previously designed other probes (Itou et al. 2013; Staudacher et al. 2016, 2018; Schmidt 2019) for other species of interest to form a multiple use probe to detect many species that predators may have fed on in one reaction, called a multiplex pcr. We formed two reactions to test for 8 different prey types (Figure 2,3). We then used this new tool for detecting predation on burrower bug and other prey and pests on field collected predators from peanut and cotton systems.

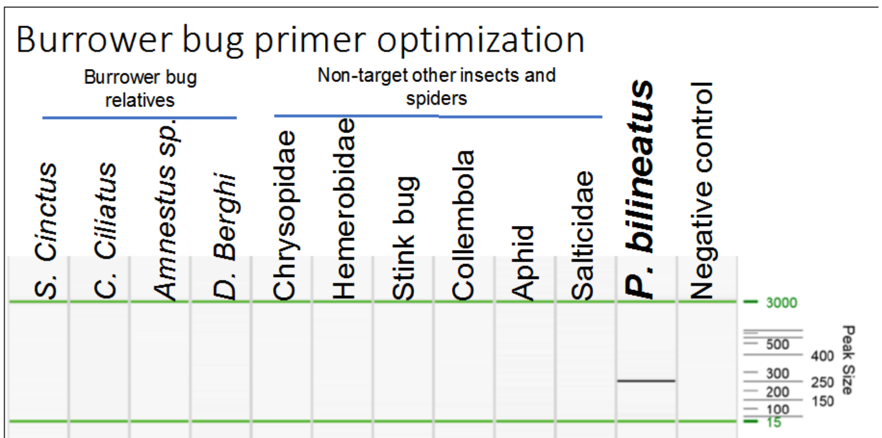


Figure 1. Using eight different temperatures, we found one temperature that had the highest, darkest line, positive test for *P.bilineatus*, the burrower bug. This probe did not result in positive tests for other species related to burrower bugs, the burrower bug relatives, or to non-target insects or spiders.

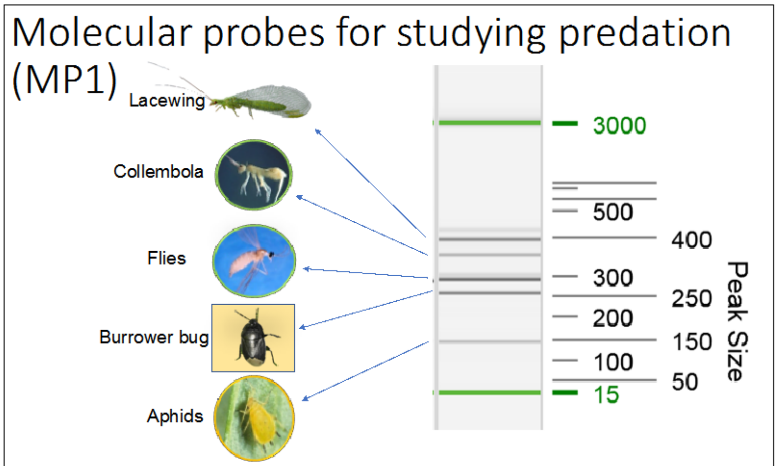


Figure 2. New burrower bug probe, highlighted in yellow box, combined with other prey types found in many systems in GA including peanut.

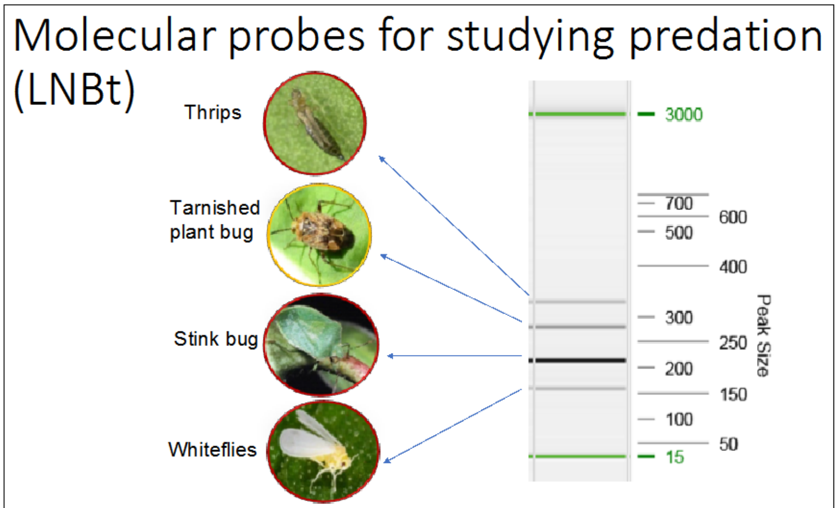


Figure 3. Second molecular probe system used that includes potential prey that sustain predators and known pests at large for multiple agricultural systems.

<u>2) Screen predators collected from commercial peanut fields around Tifton area for predation on pests (thrips, caterpillars, whiteflies)</u>

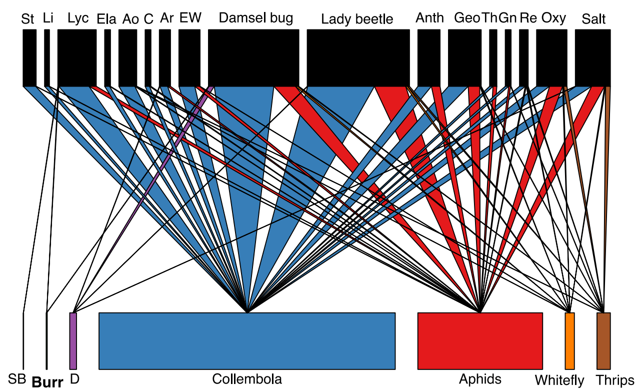
Cotton and cover crops:

After designing the burrower bug primers to test for predation on burrower bugs. We used the primers to first look at predation in a cotton cover crop experiment. These data were collected from an experiment in 2017, were we found very few burrower bugs in the pitfall traps, but in 2018, we did see quite a few burrower bugs. We haven't screened the 2018 samples for predation on burrower bugs and it will be interested to see if higher levels of burrower bug activity led to higher predation by the community of predators we observed.

For 2017, we observed 6 positive predation incidents detected on burrow bugs from 1,096 predators screened. Four of the incidents of predation were by Geocoridae (big eyed bug), one Reduviidae (assassin bug), and one Nabidae (damsel bug). Burrow bugs were only found in the arthropod community samples on two sample dates, 6/23/17 and 8/2/17, with 16 and 7 individuals collected from 36 total samples, respectively. Two of the Geocorids were from the 5/16 sample date where no burrow bugs were collected. Two Geocorids and the Reduviid positive were sampled on 6/23 which has the highest burrow bug abundance. The single nabid was collected on 8/14 where no burrow bugs were collected in field. Over the entire season, we collected and screened 95 Geocoridae, 27 Reduviidae, and 50 Nabidae. Which indicates that

Peanut open field system:

We tested the primer system for investigating feeding on burrower bug and other known pests to agriculture. During the 2017 growing season, we sampled predators from a 150 acre commercial peanut system in Tift County, GA. Samples were taken from 36 sites spaced 164 ft apart on three sample dates, 7-Jul-2017, 3-Aug-2017, and 13-Sep-2017. We observed a total of 31 different predator families, and collected over 1,200 predators on these dates. We screened 940 predators for recent feeding on aphids, burrower bug, thrips, stink bugs, and alternative prey (flies, Collembola). Of these samples, seventeen different predator families tested positive for different prey types in the field with at minimum 10 positives on prey (Figure 4). Rare predation or predators were excluded (Figure 4). Initial analysis of this peanut food web shows that few predators are feeding on burrower bug. The alternative, non-pest prey group, Collembola, appears to be fueling the predatory community, with many predators testing positive for feeding on this insect group. Collembola is a mostly soil dwelling group of insects that are found on the leaves of plants and even found up in cotton canopies. They feed on decaying material, and can be very numerous in some environments. We also see predation on stink bugs, whiteflies, and thrips. These are all good things to see in peanuts. Any biocontrol occurring in peanut prior to insects moving onto cotton is beneficial to both peanut and cotton growers. Now that this system is fully designed, we are ready to test broader scale pattern and target both the ground active community of predators and those in the peanut vegetation. This will allow for calibrating the importance of different species for maintaining biological control services in this system and help suggest which species are being removed or are absent when pests become difficult to manage.



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sustained by non-pest prey insects.

3) Determine if fire ants can be used in these studies, and if so are fire ants contributing to biological control of peanut pests. Success of this objective will lead to development of full research program on fire ants in this system.

We first assessed the quality of fire ant samples that we had collected over the last three years. Samples were organized by study system, experiment, location collected in fields or field treatments. Unfortunately, discovered that very view fire ants were collected from the open field peanut system that we sampled at 36 locations spaced 146 ft apart on three sample occasions. The best samples we found were from an open field system of dryland cotton that was approximately 120 acres. In the three years, 2015-2017, we sampled on three dates from 17 locations. In addition, we isolated fire ants from a cover crop experiment. The total number of samples that ranged between 3-20 individual ants per 3 cotton plants sampled provided over 376 samples for the cover crop experiment and open field sampling. We selected a small set of ants from samples containing many individuals. Twelve initial extractions were conducted, and then using universal primers for insects, we assessed if we could successfully amplify ant DNA following our commonly used DNA extraction method. 100% of the samples provided amplifiable ant DNA. Following this conformation, we processed the 376 high quality samples and screened them for MPI multiplex probe for prey and a caterpillar probe to look at feeding on caterpillars too. Of the 281 fire ant samples processed so far, 149 were positive for aphids, 0 for burrower bug, 16 for collembola, 7 for flies, 1 for lacewing, and 0 for caterpillars. These results suggest that this molecular probe technique can be successfully applied to study fire ant predation in open field situations, and shows they are feeding frequently on aphids and even have intraguild predation as they are feeding on lacewings. Further study using the second primer system, LNBt and spider mites will finalize this study to provide much needed information on the roles of spiders ants in biological control in peanuts and they types of prey insects supporting their populations.

References:
Itou et al. (2013) Gut content analysis to study predatory efficacy of Nesidiocoris tenuis (Reuter) (Hemiptera: Miridae) by molecular methods. Entomological Research 16:145-150.
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