NHF 2019-2020 Final Report

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Project Narrative and Justification

Florida is the number one hibiscus producing state of which most is grown in south Florida. Hibiscus is shipped throughout the North American continent. Approximately 20 to 25% of plants sold from Miami-Dade County are hibiscus. These hibiscus growers have a very narrow shipping window of 3 months in the spring of each year. The potential loss of any of this time to sell can be devastating to these growers and the Florida industry. Hibiscus is a crop that is already affected by common diseases and pests, such as bacterial leaf spot, two-spotted spider mite, among others. However, more recently, a newly introduced pest has become the major concern for nursery growers. The hibiscus bud weevil HBW (*Anthonomus testaceosquamosus*) has been reported as a pest of hibiscus in Texas and Mexico for several decades but was not detected in Florida until May of 2017 when it was found in the Miami-Dade area. Currently, the hibiscus bud weevil has been also reported in Broward, Hernando, Collier, Seminole, and Duval Counties.

As of now, any grower found with this weevil cannot sell until the weevil is controlled and can no longer be found. Growers are at a loss of what steps are necessary to control this pest and which, if any, of the insecticides can control the pest. Although this pest, is a native to Texas and/or north Mexico, there has been very little research conducted on the management of this pest. The goal of this research was to identify and test the tools hibiscus growers need to manage the HBW such that there is no down time during the 3-month sell window. Although this pest is in the same genus as several other key pests (i.e. pepper weevil, cotton boll weevil), there is no information about its biology and life cycle. The proposed research was divided into three main objectives: A. Basic biology, rearing and sampling; B. Management with insecticides and other inputs; and C. Alternative management strategies.

A. Basic biology, rearing and sampling

Basic biology

We were interested in studying the life cycle of HBW at 27° C because preliminary studies showed that this is an optimum temperature for development. We were also interested in exploring whether we could use an artificial diet to rear this pest, therefore we also studied the biology of the HBW at 27° C using the pink bollworm diet (*Pectinophora gossypiella*, Lepidoptera: Gelechiidae). Lastly, the life cycle of HBW was also studied at 34, 15 and 10° C to explore how its development is affected by low or high temperatures.

Methods: Hibiscus buds (*H. rosa-sinensis* var. Painted Lady) were used to estimate the HBW egg to adult development in its natural habitat under different temperatures. Twenty eggs (24h-old)

were randomly selected and inserted individually into a new non-infested bud. Each egg was placed on the anthers by creating a rectangular opening on the bud. The buds were then placed individually in petri dishes. The petri dishes were kept in an incubator at 10, 15, 27 or 34 ± 1 °C, 12:12 h L:D and 60% RH. The buds were inspected daily and each day the developmental stage of the weevil present was recorded. The experiment stopped until all individuals reached adulthood or died before that.

To estimate population growth and adult longevity under favorable (27±1 °C, 12:12 h L:D and 60% RH) conditions, twenty pairs were randomly selected and offered a hibiscus bud as feeding and oviposition substrate. Daily oviposition was scored for each female and adults were observed until death. Longevity was calculated for both males and females.

To test whether HBW can develop and reproduced on an alternative diet, the diet for mass rearing of the pink bollworm was used. Diet was placed and compressed into 24-well cell culture plates and 70 eggs were individually placed in each well. To simulate the bud conditions, plates were kept in the dark until adult emergence at 27 ± 1 °C and 60% RH. Eggs were monitored daily and larval feeding on the diet was confirmed by the lightly pink colorization of their body.



Figure 1. Tissue-culture plate with compressed artificial diet of the pink bollworm.

Upon adult emergence females were paired with males. Twenty pairs were randomly selected and offered a hibiscus bud as feeding and oviposition substrate and twenty pairs were offered the artificial diet. All adult pairs were kept at 27 ± 1 °C 12:12 h L:D and 60% RH. Daily oviposition was scored for each female and adults were observed until death. Longevity was calculated for both males and females. To estimate the sex ratio of the progeny, six egg cohorts were created, by individually isolating all the oviposited on the buds eggs in cell culture plates containing diet. Eggs were reared to adulthood when the gender of each individual was scored. All plates were kept in dark at 27 ± 1 °C and 60% RH.

Table 1 provides a summary of the development of HBW. At 27°C, eggs hatch within 2-3 days. The larval stage has three instars and can last, 6.5-10 days. The pupal stage lasts 2.9-4.2 days. The egg to adult development can range from 12.9-16.5 days, and survival can be up to 90%. Adult longevity ranges from 11-127 days, with the males living longer than the females. The sex ratio is 1:1 females to males. Very low or high temperatures appear to be detrimental to the development of this weevil. At 10°C, no eggs hatched, while at 15°C, eggs hatched 11 days after oviposition,

but the larvae did not feed and eventually died. Similarly, at 34°C, eggs hatched within 5.6 days, but none of the larvae managed to pupate.

Table 1. Duration (in days) of each developmental stage of the hibiscus bud weevil in different temperatures and food source.

Food source	Temperature (°C)	Egg	Larva	Pupa	Egg- Adult	Female	Male
	10	76.4	NA	NA	NA	NA	NA
II:h: hd	15	11.15	2.19	NA	NA	NA	NA
Hibiscus bud	27	3.45	6.5	2.9	12.85	48.9	79.1
	34	5.55	15.4	NA	NA	NA	NA
Pink bollworm diet	27	2.22	10.05	4.21	16.49	72.7	61.1

Although the weevils could develop and reproduce on the artificial diet of the pink bollworm, the hibiscus buds were a superior food source that allowed for higher daily oviposition and therefore higher population growth. Table 2 provides a summary of the life history parameters calculated based on the daily oviposition of the weevils. Note that for this experiment three groups of weevils were used. The first group was developed and reproduced on hibiscus buds, the second group was developed and reproduced on the pink bollworm diet and the third group developed on the pink bollworm diet and reproduced on hibiscus buds.

Table2. Population growth (rm), net reproductive success (Ro), generation time (GT), doubling time (DT), finite rate of increase (λ) and oviposition rates (eggs/Female/day) for *Anthonomus testaceosquamosus*, when it weevils developed solely on buds, or solely on diet or on diet as juveniles and bud as adults.

Food source	rm	Ro	GT	DT	λ	Oviposition rate	N (#females)
Hibiscus bud	0.49	138.42	10.02	1.41	1.64	5.85	20
Pink bollworm diet	0.06	7.48	35.30	12.16	1.06	0.73	21
Bud + Diet	0.09	21.85	36.01	8.09	1.09	0.2	20

Rearing

Infested buds with weevil larvae were initially placed outdoor in a plant cage with a hibiscus plant (var. Painted Lady). Upon adult emergence, a new plant was placed in the cage weekly. Fallen buds were collected weekly and placed in a plastic container. Containers were kept in the laboratory at room temperature approx. 24° C. The newly emerged adult weevils from the container were subsequently transferred back to the plant cage to infest a new hibiscus plant. This process was followed for a month and a half, after which a large reduction in the dropped/infested buds in the cage was observed. Therefore, the colony was maintained in incubators at 27 ± 1 °C, 12:12 h L:D and 60% RH using the plastic containers. Fresh buds were offered to the adult weevils in the containers weekly, while the old buds were removed and maintained in isolation until adult emergence. Although female weevils oviposit multiple eggs per hibiscus bud, only 2-3 individuals can reach adulthood due to the high larval cannibalism. Hence, to expand the colony individual eggs were isolated to individual buds that were then placed in petri dishes. Emerging adults from these buds were transferred to a new container to feed and reproduce on fresh hibiscus buds.

Upon increase of the weevil population, the weevils were transferred in cages in groups of 100-150 individuals together with hibiscus buds. The buds were replaced twice per week. The old buds were held separately in cages until adult emergence. Although this method was less labor intense, in comparison to egg separation, it was not 100% reliable. Weevil populations were fluctuating, and massive deaths were scored in the rearing cages. Deaths derived either from aging or killing. Due to the unbalanced sex ratio in cages many adult males were found decapitated and in many cases without legs. For these reasons we adjusted the rearing method using smaller groups of weevils (35 weevils total) with a sex ratio of 2:1 females:males and two buds per weevil ratio. We also explored whether using the artificial diet of pink bollworm could assist in the rearing of the hibiscus bud weevil. The diet could bypass the use of buds in the juvenile stage and could increase the individuals that reach adulthood. To use the diet in the rearing process the eggs had to individually be isolated in the tissue culture plates (see photo above) therefore eliminating larval cannibalism.

Decreasing the total number of weevils per cage and switching to a female biased sex ratio increased the number of offspring. The use of the artificial diet, however, did not assist in the rearing. Weevils that developed on the diet, did not produce as many offspring as weevils that developed in buds. Therefore, we concluded that the best way to maintain the hibiscus bud weevil colony is by using a group of maximum 35 weevils per cage with 70 buds and a sex ratio of 2:1 females to males.

A manuscript describing the biology of the hibiscus bud weevil under different temperatures and different food regimes is being prepared. An extension publication (EDIS) has been submitted and is currently under review.

Sampling

Three nurseries were sampling and providing us with hibiscus buds weekly for six months. The aim of these samplings was to identify natural enemies that could potentially be used a the management program. In two out of three nurseries a parasitoid of the genus *Catolaccus* (Hymenoptera: Pteromalidae) was detected. Unfortunately, only three speciemens were collected that were not sufficient for identification and rearing. Future efforts will focus on collecting, identifying, rearing and test this parasitoid against the HBW.

B. Management with insecticides and other inputs

1) Evaluate contact foliar insecticide sprays for adult control

Methods: Hibiscus rosa-sinensis 'painted lady' plants in 3-gallon containers with buds were selected for the test. Test plants were sprayed with the insecticides to thoroughly cover the foliage and buds. Control plants were sprayed with water. After the insecticide dried, buds and leaves were removed and placed in petri dishes in the laboratory. Leaves and buds were placed in separate petri dishes and two adults were placed in each petri dish. Weevil mortality was recorded at 4, 24, 48, 72, and 96 hrs. after application. In the direct exposure test, weevils were sprayed directly with the insecticide treatment.



Figure 2. Petri dishes with buds or leaves and weevils.

The insecticides tested for adult control included the following. All products were used at the high rate for weevil/beetles:

- Acelepryn (chlorantraniliprole)
- Acephate (acephate)
- AzaSol (azadrachtin)
- Conserve (spinosad)
- Marathon (imidacloprid)

- Pylon (chlorfenapyr) (off-label)
- Mainspring GNL (cyantraniliprole)
- Sevin SL (carbaryl)
- Talstar (bifenthrin)
- Xxpire (sulfoxaflor + spinetoram)

Mortality of adult weevils after feeding or walking on treated plants was relatively low for all products tested. Xxpire provided the best result with 40% mortality of adults feeding or walking on buds and leaves (Tables 3 and 4). Benefit (imidacloprid) provided similar results on <u>leaves but only</u> killing 35% of the weevils. Direct sprays caused more mortality than residual/feeding exposure. Talstar caused 90% mortality followed by Conserve with 50% mortality (Table 5).

Although Pylon provided moderate mortality for both residual/feeding and direct contact, this product is not recommended because it is not labeled for outdoor use.

Table 3. Residual/Feeding mortality from sprayed buds - adults walked on and fed upon buds that were sprayed.

Product	Active Ingredient	Rate	% Adult Mortality after 4 days
Xxpire	Sulfoxaflor+spinetoram	0.08 oz/3 gal	40
Pylon	chlorfenapyr	6 oz/ 100 gal	30
Conserve SC	spinosad	0.06 fl oz/gal	15
Orthene 97	acephate	12 oz/100 gal	10
Sevin	carbaryl	1 qt/100 gal	10
AzaSol	azadirachtin	6 oz/50 gal	10
Acelepryn	chlorantraniliprole	16 oz/100 gal	10
Talstar P	bifenthrin	21.7 oz/100 gal	0
Control	water	NA	0
Mainspring GNL	cyantraniliprole	8 oz/100 gal	0
Benefit 60WP	Imidacloprid	0.71 oz/100 gal	0

Table 4. Residual/Feeding mortality from sprayed leaves - adults walked on and fed upon leaves that were sprayed.

Product	Active Ingredient	Rate	% Adult Mortality after 4 days
Xxpire	Sulfoxaflor+spinetoram	0.08 oz/3 gal	40
Benefit 60WP	Imidacloprid	0.71 oz/100 gal	35
Talstar P	bifenthrin	21.7 oz/100 gal	25
Pylon**	chlorfenapyr	6 oz/ 100 gal	25
Conserve SC	spinosad	0.06 fl oz/gal	25
Sevin	carbaryl	1 qt/100 gal	25
Orthene 97	acephate	12 oz/100 gal	20
Control	water	NA	5
Mainspring GNL	cyantraniliprole	8 oz/100 gal	5
Acelepryn	chlorantraniliprole	16 oz/100 gal	5
AzaSol	azadirachtin	6 oz/50 gal	0

^{**}Off label use

Table 5. Adult mortality from contact spray directly to the adult

Product	Active Ingredient	Rate	% Adult Mortality after 4 days
Talstar P	bifenthrin	21.7 oz/100 gal	90
Conserve SC	spinosad	0.06 fl oz/gal	50
Pylon**	chlorfenapyr	6 oz/ 100 gal	40
Xxpire	Sulfoxaflor+spinetoram	0.08 oz/3 gal	30
Orthene 97	acephate	12 oz/100 gal	30
Sevin	carbaryl	1 qt/100 gal	30
Control	water	NA	30
Mainspring GNL	cyantraniliprole	8 oz/100 gal	30
AzaSol	azadirachtin	6 oz/50 gal	20
Benefit 60WP	Imidacloprid	0.71 oz/100 gal	20

Acelepryn	chlorantraniliprole	16 oz/100 gal	10	

^{**}Off label use

2) Evaluate systemic insecticide drenches for larval control

Five systemic insecticides were tested as a drench treatment for their impact on the presence of live weevil larvae and eggs within buds and the number of oviposition and feeding holes on buds. Flagship provided the best control in reducing the number of live larvae, eggs, and holes. This trend was seen with both buds on the plants and on dropped buds. No product provided 100% control.

1. A total of 24 plants (4 plants per treatment) (6-inch pot Painted Lady Hibiscus) were drenched with 200ml of each insecticide solution based on the rates below on February 21, 2020.

Table 6. Rates and solutions prepared for each systemic insecticide

Product	Active ingredient	Rate	Solution (SI)
Benefit 60WP	imidacloprid	20 g/100 gal	53 mg/L
Safari	dinotefuran	24 oz/100 gal	1.8 g/L
Flagship	thiamethoxam	8.5 oz/100 gal	0.63 g/L
Altus	flupyradifurone	3.7 fl oz/100 gal	289 μl/L
Mainspring GNL	cyantraniliprole	8 fl oz/100 gal	625 μl/L
Water	NA	NA	NA

- 2. 10 days <u>after</u> drenching, the plants were placed in cages with 20 adult weevils March 3, 2020
- 3. 1st evaluation 7 days after weevil release (March 10, 2020) five buds were removed from each plant and evaluated for the number of holes, number of eggs, and number of live larvae in the buds. All dropped buds within each cage with 4 plants were also collected and evaluated for live larvae.
- 4. 2nd evaluation 7 days after 1st evaluation (March 17, 2020) remaining buds were removed from each plant and evaluated for the number of holes, number of eggs, and number of live larvae or pupae. All dropped buds around the plants were also collected and evaluated.

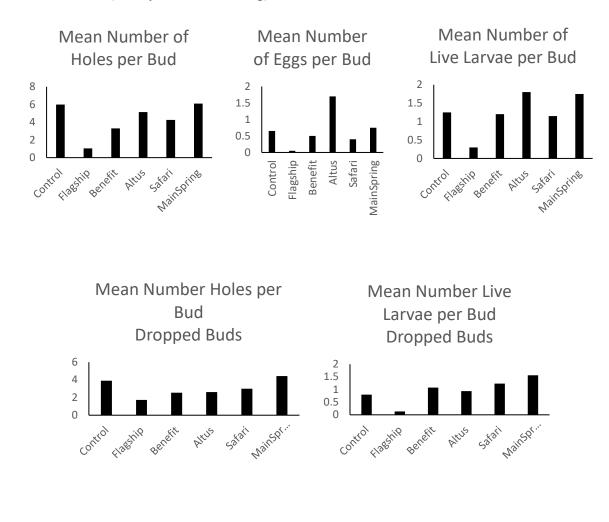
Results

Of the 5 products tested, Flagship (thiamethoxam) provided the best results in that there were fewer holes on the buds, and fewer eggs and larvae in the buds. No product provided complete (100%) control. During the first evaluation, on average there were 3 to 6 holes per bud, except for Flagship which had 1 hole/bud; there were on average 0.5 to 1.5 eggs per bud, except Flagship which had 0.05 eggs per/bud; and the average number of live larvae per bud was 1.25 to 1.8 per bud, except Flagship which was 0.3 per bud. (see graphs below)

A similar trend was also noted for the dropped buds in which the average number of holes ranged from 2.5 to 4.4, except Flagship which was 1.7 holes/bud. The average number of larvae in the dropped buds ranged from 0.8 to 1.6, except Flagship which was 0.1 larva per bud.

On the second evaluation 7 days after the first, there were fewer buds on the plant to evaluate so the overall number of buds evaluated varied among the treatments. The number of live larvae in buds averaged 0.09 to 1.3 per bud, except for Flagship which averaged 0.4 larvae per bud. The reduction of larvae in buds treated with Flagship may also be because there were less eggs laid in these buds and not actually killing the larvae. The adult weevils may be deterred and therefore there is less feeding and egg laying, resulting in fewer larvae. This poses the question if Flagship is sprayed as a foliar application, will it provide the same results as with the drench application? This active ingredient is commonly used as a foliar application against pepper weevil.

First Evaluation (14 days after drenching)



Second evaluation (21 days after drenching)

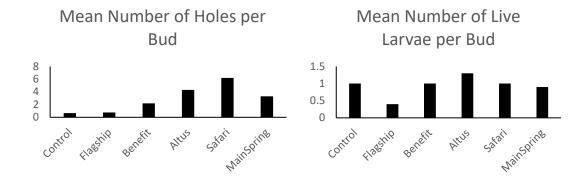


Figure 3. First and second evaluation of systemic insecticides against the hibiscus bud weevil.

A follow up experiment to test Flagship as a foliar insecticide was conducted. The same methods as described above were followed. Efficacy of Flagship was evaluated on buds, leaves and directly on adult weevils. Additionally, daily oviposition and feeding holes were scored (for 4 days) on sprayed buds, to explore whether Flagship deters weevils from oviposition and feeding.

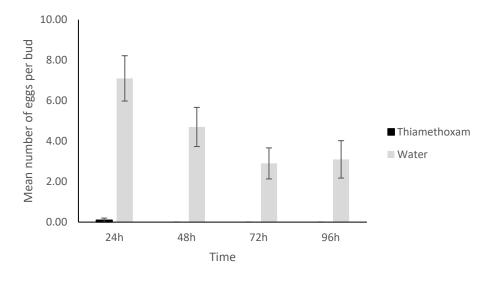
Table 7. Rates and solutions prepared to evaluate the efficacy of Flagship as a contact insecticide.

Common name	Active ingredient	Rate	Solution (SI)
1. Flagship	Thiamethoxam	8 oz/100 gal	0.6 gr/L
2. Water	NA	NA	NA

Table 8. Mortality of hibiscus bud weevil when Flagship was applied on the buds, leaves and directly on adult weevils.

Common name	Active ingredient	% Mortality on buds	% Mortality on leaves	% Mortality of adult weevils
Flagship	Thiamethoxam	30	40	40
Water	NA	0	0	0

When Flagship was applied as a contact insecticide had similar efficacy to other contact active ingredients. Furthermore, Flagship deterred adult females from oviposition and feeding (Figure 4). Almost no eggs were oviposited within 4 days, while feeding was minimal in comparison to the control. These results answer the posed question from "Evaluate systemic insecticide drenches for larval control which includes determining if the insecticide can get into the bud" and demonstrate that Flagship can provide equally good results when used as a foliar contact insecticide.



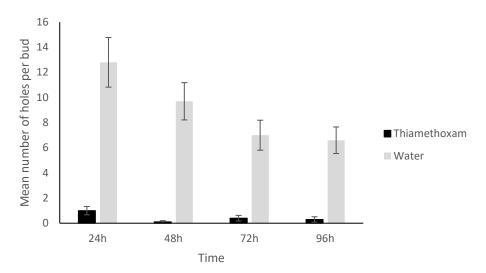


Figure 4. Daily oviposition and feeding of adult weevils when offered buds sprayed with Flagship or water. Note that buds were refreshed daily by taken from sprayed plants. Plants were sprayed only once at the start of the experiment; therefore, the results show the residual activity of Flagship on buds after 4 days.

3. Evaluate Entomopathogenic nematodes for larval control, which includes determining if the nematodes can get into the bud

Three entomopathogenic nematode products were tested under laboratory conditions for larval control on *Hibiscus rosa-sinensis* 'painted lady'. These products included Nemasys G (*Heterorhabditis bacteriophora*), Nemasys (*Steinernema feltiae*) and Millineum (*Steinernema carpocapsae*). All three nematode products show promise as a management tool for this weevil. In all the laboratory tests, Nemasys and Millineum provided 100% mortality and Nemasys G provided 90% control in one of the tests. This demonstrates that the nematodes can kill the larvae and are capable of entering buds where the larvae reside and feed. However, in a foliar test in

which the nematodes were sprayed onto the buds simulating a field application, the results were not as good. How these nematodes are applied will be essential to their success in killing weevil larvae and studies will continue to examine this issue.

- Laboratory Procedure Test 1
 - Slightly opened buds
 - Placed two 20ul droplets with nematodes on bud (approximately 200 nematodes/bud)
 - Four days later, buds were opened to determine live and dead larvae

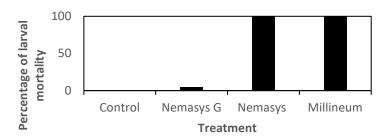


Figure 5. Percentage of larval mortality when buds were slightly opened.

- Laboratory Procedure Test 2
 - Larvae were removed from the buds
 - Nematodes were placed directly on the larvae (approximately 200 nematodes/larva)

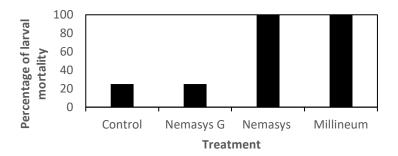


Figure 6. Percentage of larval mortality of exposed larvae.

- Laboratory Procedure Test 3
 - Closed buds
 - Placed two 20ul droplets with nematodes on bud (approximately 200 nematodes/bud)

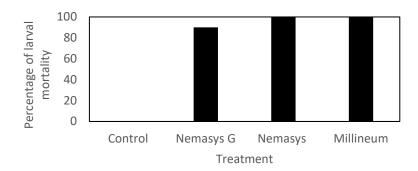


Figure 7. Percentage of larval mortality in closed buds.

Foliar-applied nematode test:

- Infested 20 plants within a large screen cage for one week
- Plants were removed from the cage and nematode applications were made (5 plants per treatment)
- Nematodes Steinernema feltiae (Nemasys) and Steinernema carpocapsae (Millineum)
 - Applied approx. 800,000 nematodes to 5 plants (160,000 per plant) which is very high rate (2-3 times recommended rate)
 - Nematodes were applied in 1 gal water to the 5 plants
 - All buds were removed 4 days after application and examined for live/dead larvae

Table 9. Summary of the greenhouse experiment when nematodes were applied on hibiscus plants infested with the hibiscus bud weevil.

	# Buds	# Holes	# Eggs	# Larvae	# Live Larvae	# Dead Larvae	% Dead
Control	44.8	17.8	0.8	9.2	9.2	0	0
Nemasys	44.6	28.0	3.6	10.4	9	0.6	6.2
Millineum	45.4	14.2	0.4	4.4	3.8	0.6	13.6

4. Evaluate Entomopathogenic fungi for adult control

Five entomopathogenic fungi products were tested under laboratory conditions for adult weevil mortality feeding on *Hibiscus rosa-sinensis* 'painted lady'. All but one product provided good control (>70%) when the weevil was sprayed directly and moderate to low mortality (10-40%) when the weevil was placed on foliage/buds that had been sprayed with the product.

Table 10. Rates and solutions prepared of different entomopathogenic fungi.

Treatment	Active ingredient	Rate	Solution (SI)
Botanigard 22WP	Beauvaria bassiana Strain GHA	2 lbs/100 gal	2.4g/L
Botanigard ES	Beauvaria bassiana Strain GHA	2 qt/100 gal	5ml/L
BioCeres WP	Beauvaria bassiana Strain ANT-03	3 lbs/50 gal	7.2/L
NoFly	<i>Isaria flumosorosea</i> strain FE 9901	16 oz/100gal	1.2g/L
Velifer	Beauvaria bassiana strain PPRI 5339	900ml/1000L	900ul/L
Control (water)	NA	NA	NA

Residual/feeding test: One hibiscus plant (6-inch pot) was sprayed with a treatment solution with a hand-held pump sprayer for complete coverage of the buds and leaves of the plant (approximately 250 ml). Ten stems with at least one bud and leaf were removed from each plant one hour after application. The stem was placed in a deep petri dish (100 x 40 mm) with a hole to insert the stem. The petri plate was placed on top of a cup of water so that the stem remained in water. Two adult weevils were placed in each petri dish. The weevils were able to move freely within the petri plate walking and feeding on the leaf and bud. 10 replications (bud/leaf stem) with 2 weevils per replication; completely random.



Figure 8. Experimental set up used for evaluation of entomopathogenic fungi.

<u>Direct contact test</u>: 10 randomly selected weevils were placed in a deep petri dish (100 x 40 mm) with 2 pieces of filter paper. The filter paper absorbs excess solution, so no weevils were sitting in "puddles" of solution. The dish was briefly opened, and 3-4 pumps of a treatment solution was sprayed directly on the weevils within the petri dish. Ten adults were directly sprayed so each weevil was completely covered by the product solution. The weevils remained in the petri plate until death. One or two hibiscus leaves were placed in each plate for food and moisture. Ten replications; completely random.

Weevils were monitored 2, 4, 7, and 10 days after treatment for mortality and signs of infection (mycosis). Any dead weevils were held in moist chambers to determine if cause of death was due to the fungal product.

Results:

Most of the products tested provided good control when sprayed directly on the weevils (70-100% mortality), except for NoFly (~20%mortality) (Fig. 9). The. However, the mortality rate was less when the adults were exposed to leaves and buds sprayed with the products and contact was from the interaction on the plant and not a direct spray (Fig. 10). Note that the conditions of this test provided a desirable controlled environment for the products to work and results in the field may differ.

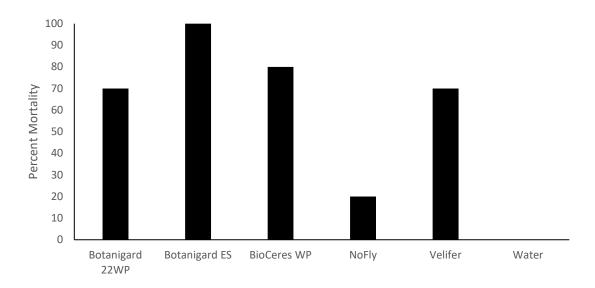


Fig. 9. Mortality of adult weevils sprayed directly with the product and held in a humid environment.

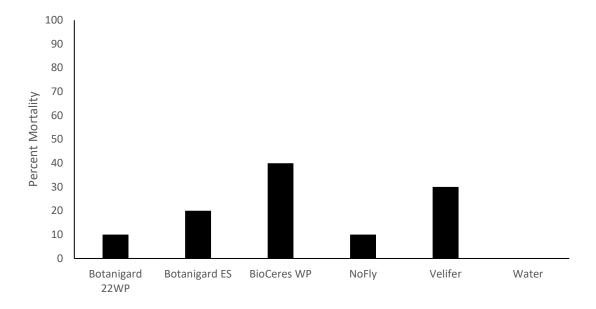


Fig. 10. Mortality of adult weevils exposed to sprayed foliage/buds.

C. Alternative management strategies

Several species in the *Anthonomus* genus are attracted to a group of commercial lures that consist of male aggregation pheromones and host plant volatiles. There are four components of the synthetic male aggregation pheromone, also known as Grandlures (I-IV). In a laboratory experiment we tested for attraction to the cotton boll (*A. grandis*), and pepper weevil (*A. eugenii*) lures. We used plastic arenas where on one end we placed the lure and on the other end we placed a blank filter paper (control) (Figure 11). We then released a single adult male weevil and observed

towards which end it would go. Each individual was observed for 2h and in total we tested 16 individuals for each lure.



Figure 11. Plastic arena used to test attraction to commercially available lures of boll and pepper weevil. Adult males were released in the center of the arena and were free to choose between the lure or the blank filter paper (control).

Male weevils appeared to be more attracted to the pepper weevil than the cotton boll weevil lure (Table 11). In the case of the cotton boll weevil lure, male weevils did not show a preference.

Table 11. Results of two-choice experiments. Male weevils could choose between a lure or a blank filter paper (control).

Tested Lure	Tested Males	% Choosing Lure	% Choosing Control
Cotton boll weevil	16	50	50
Pepper weevil	16	63	37

Yellow sticky traps are the most attractive traps for several *Anthonomus* species. Preliminary tests at Tropical Research and Education Center showed that yellow sticky traps have more potential in attracting the HBW than the typical cotton boll weevil traps. Field trials are currently being

conducted to test for attraction to the boll, and pepper weevil lures and to identify the best trap to catch adult hibiscus bud weevils. Three locations (nurseries) have been selected to test the attraction of hibiscus bud weevil to the cotton boll or pepper weevil lure in combination with yellow sticky traps. Figure 12 shows the set up currently being tested in the field. Traps with lures or without (control) are being rotated weekly to account for the different position. Every week the yellow sticky traps are replaced with new ones and every three weeks the lures are also replaced.



Figure 12. Experimental set up used to test attraction of hibiscus bud weevil to the cotton boll or pepper weevil lure in combination with yellow sticky traps. The plate offers protection from the water because the traps are attached to the sprinkler rebars.

Table 13. Locations and number of replicates per treatment in each nursery.

Location	Treatment	Replicates
Nursery 1	Cotton boll weevil lure	
	Pepper weevil lure	10
	No lure (control)	
Nursery 2	Cotton boll weevil lure	
	Pepper weevil lure	10
	No lure (control)	
Nursery 3	Cotton boll weevil lure	5
	Pepper weevil lure	
	No lure (control)	

So far, no weevils have been caught in any of the traps. This is not an unexpected result as weevil populations are very low during this period, due to the lack of buds and reduced foliage of the plants. Weevil population is generally high between March and July. Moreover, this November has had a lot of precipitation and recently an incident of the Tropical Storm Eta. Experiments will run throughout the year to explore whether we can use the lures as a monitoring tool.

Disclaimer

Due to the unforeseen incidence of COVID-19 the UF-TREC remained closed from mid-March through the end of May. During this period only essential activities were allowed. These measures resulted in a decrease of the HBW colony and delays in the proposed research plan. In addition, the previous PI of this project Dr. Catharine Mannion retired at the end of August (2020) and the current PI became responsible for the deliverables of this grant. Several experiments are still underway and despite these unexpected changes, are expected to be completed by January 31, which is the project expiration date.