FINAL PROJECT REPORT

THE EFFICACY OF THE FUNGUS, BEAUVERIA BASSIANA, AS A BIOLOGICAL CONTROL AGENT AGAINST THE SWEET POTATO WEEVIL (*Cylas formicarius*) ON SWEET POTATO (*Ipomoea batatas*) IN JAMAICA

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Methodology

Culturing of *Beauveria bassiana*

Pure culture of *Beauveria bassiana* on rice was obtained at Bodles Research Station which was stored at approximately 7^{o}C in the refrigerator at the Plant Pathology Laboratory. Approximately 1361g (3 lbs) of parboiled rice was obtained and sterilized in a 500mL conical flask then autoclaved.

Culturing of *Beauveria bassiana* on rice

Under the laminar flow, spores from the pure culture were suspended in distilled water. To each 200g parcel of sterilized rice, the spore suspension was poured and agitated thoroughly by hand. This was done in the autoclavable plastic bags as this made it easier for the spores to be distributed evenly to each grain of rice. This bag was then folded and stapled as to properly secure the contents.

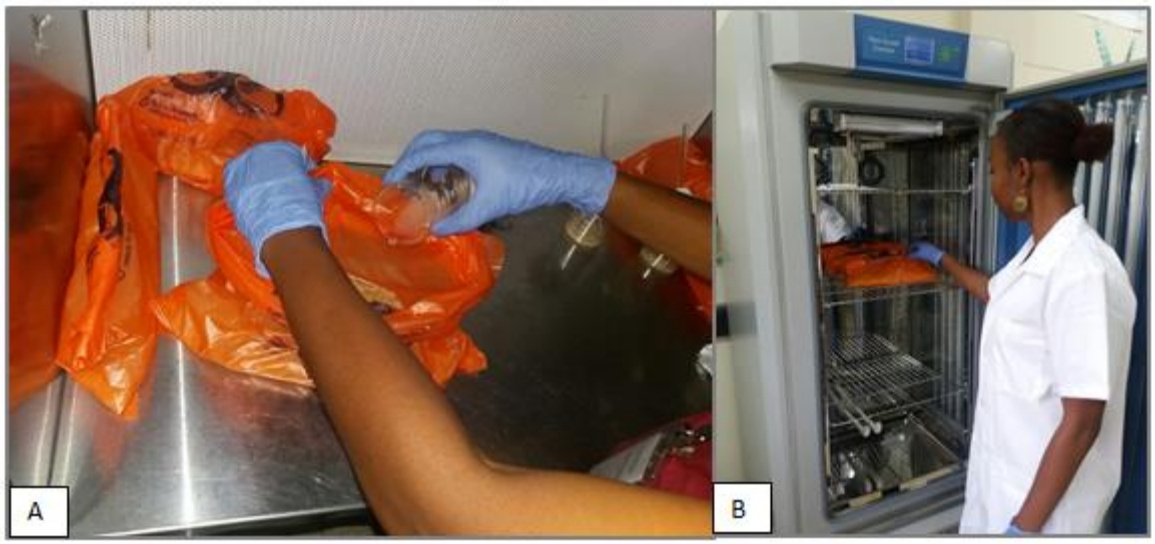

Figure 1A; Inoculating parboiled rice with *Beauveria bassiana* suspension in autoclavable bags under the laminar flow. ***1B***; Placement of the inoculated media in sealed autoclavable plastic bags for incubation at 24^{o}C and 85% Relative Humidity in the Plant Growth Chamber at Bodles Research Station.

Incubation and Storage

Incubation of the *B. bassiana* on rice was done using the Plant Growth Chamber in the Entomology laboratory which was set to 24^{o}C and 85% Relative Humidity. After 10 days, the rice was removed from the Plant Growth Chamber and transferred to brown paper bags under the laminar flow. The spores were allowed to dry for the next 10 days in the paper bags at the same 24^{o}C and 85% RH. Bags were stapled to secure the contents and prevent contamination. Parboiled rice with spores of the *Beauveria bassiana* fungus was stored in sterilized cups with lids in the refrigerator at approximately 7^{o}C until it was ready for use. The transfer of *B. bassiana* on rice from one container to another was done using aseptic techniques under the laminar flow.

Figure 2; Inoculated rice transferred from autoclavable plastic bags to brown paper bags for drying at 24^{o}C and 85% RH in the Plant Growth Chamber.

Figure 3; Dried *B. bassiana* in rice placed in sterilized plastic cups with cover.

Soil Sampling

Soil samples were collected at three different times during the trial. The first soil sample was collected on July 10, the second on September 23 and the third collected on November 20, 2017. These samples were sent to the Plant Pathology and Nematology laboratories at Bodles Research station for analyses. The objective of the first sampling was to ascertain the different microorganisms in the soil; such as beneficial or harmful fungi, bacteria and nematodes before planting of the crop. The second soil sample was collected about half way through the trial (day prior to the second application of *Beauveria bassiana*) with the aim of checking for the presence of the *B. bassiana* in the trial area and also to quantify its presence. The sample was also tested for the presence of Nematodes. The third sample was collected at the end of the trial, after final harvest of tubers and vines, to check for the presence of the *B. bassiana* to quantify its presence. This sample was also tested for the presence of Nematodes.

Soil samples for nutrient testing were collected on January 25, 2016 and the result was decided to be valid for this trial which is in the same location from which the samples were taken. A special Beauregard blend (fertilizer) was developed by Fersan to supplement the deficiencies of the plot.

Field Preparation

Land Preparation was done according to guidelines in the IICA November 2015 Beauregard Sweet Potato Cropping Calendar. Carbaryl was added to the water tank before it was mounted onto the stand for convenience. It was then evenly distributed throughout the plot area through the drip lines in a gravity fed system.

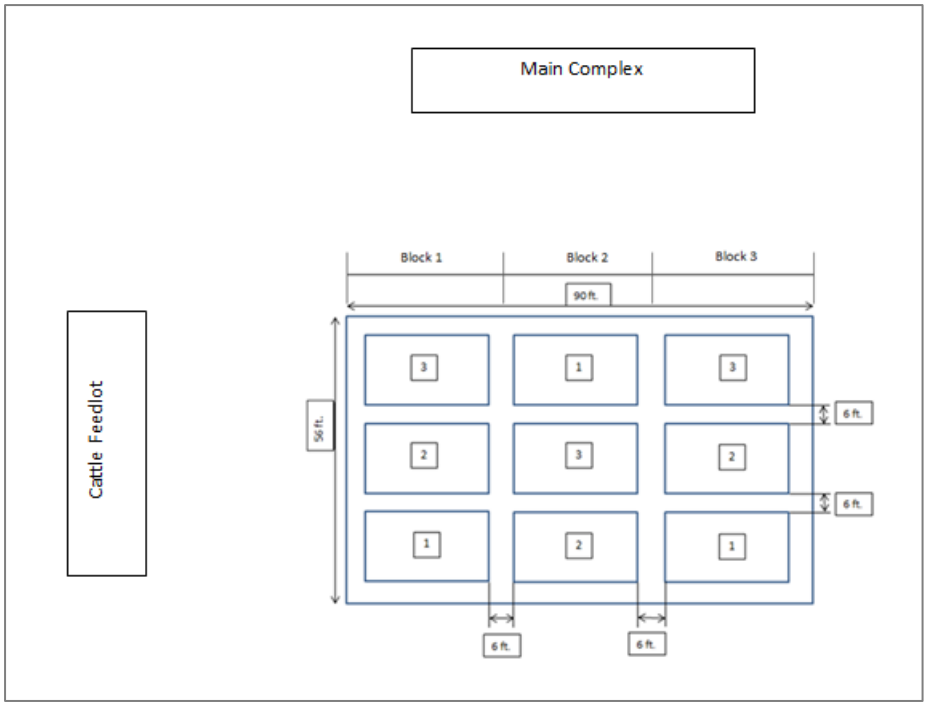

Figure 4; Layout of the research plot in relation to landmarks; Main Complex and Cattle Feedlot.

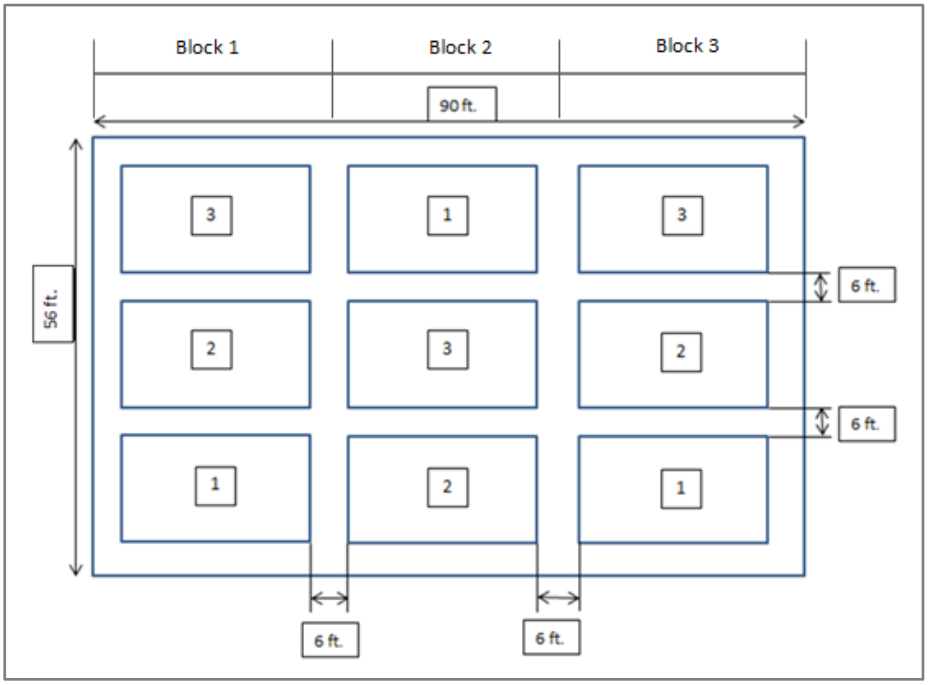

Figure 5; Layout of the research design- Completely Randomized Design. (1- No treatment [control], 2- application of the *Beauveria bassiana* at bed preparation only, 3- application of *B. bassiana* both at bed preparation and at moulding 6 weeks after planting).

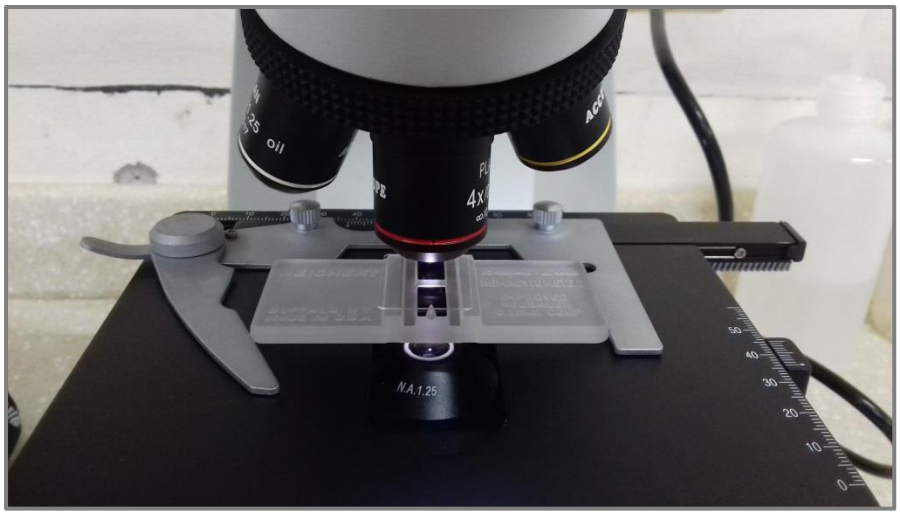

Figure 6; Haemocytometer used to estimate the number of viable spores of *Beauveria bassiana* in suspension.

The haemocytometer borrowed from the Life Sciences Department, The University of the West Indies, Mona was used to estimate the number of spores that were produced on the parboiled rice under aseptic and controlled environment in the laboratory at Bodles Research Station. The cell suspension (1:10; 1 gram or rice to 10mL water) was counted in triplicate and found to contain 8.5×10^{5} spores. Botanigard, a commercial product with *B. bassiana* as the active ingredient contains 2×10^{13} spores per pound. This experiment aimed to use approximately 15% more spores than that used in the commercial product as no stabilizers were added to extend the life of our *B. bassiana* which was applied in the natural form.

Figure 7; *B. bassiana* on rice was strained into the knapsack sprayer.

The entomopathogenic fungus on rice was placed into a gallon bottle with the required amount of water and vigorously shaken for about 10 minutes to dislodge the spores which were now growing on the grains of rice. It was then strained into the knapsack sprayer as to eliminate larger residues from clogging the nozzle. Application of the *B. bassiana* was a lengthy process as the cone nozzle was used. For future repeat of this experiment, it is recommended to use the fan nozzle which emits larger droplets and reduce the time taken for application of the *Beauveria*.

Figure 8A; Holes for planting of slips were made using a stick with measurements; ***8B;*** Dr. Johnson teaching workers the correct method of mixing the 'Plant Start' in a 50 gallons drum.

Using the stick was a more precise and consistent way of creating the holes for planting of the sweet potato slips. This stick had a mark which indicated that the hole should be dug 5 inches deep. They were then dug 12 inches apart along the row. The holes were narrow enough which provide for easy and firm compaction of soil around the slips which will better facilitate water and nutrient uptake than if holes were dug too wide. This also provided consistency and removed biases from planting of slips.

Figure 9A; First application of *B. bassiana* evening before planting; ***9B;*** Second application of *B. bassiana* about mid-way the cropping cycle

Both applications of the *B. bassiana* were applied in the evening when the time was cool. This time better aided the survival of the spores as they favour the cool and moist environments. Coincidentally, there was a light shower of rain after each application. Figure 9A was done the evening before the slips were planted so the soil was bare and left open to interaction with environmental factors. Figure 9B showed vegetative growth as this was done about half way through the crop.

Figure 10A; Undesirable Uplifter slips with brown nodes; ***10B;*** Healthy Uplifter slips with active, green nodes.

The Uplifter slips were cut from the Germplasm located at Pasture 33a which is located at Bodles Research Station. This germplasm was old, hence the slips received were not of good quality as the Beauregard which were grown from tissue culture and placed in the greenhouse. The slips in Figure 10A were old, hardened and began to lose moisture. The meristematic regions where feeder roots develop were dying and thus not selected for planting on August 21.

Figure 11A, 11B; sweetpotato field properly maintained with corn as barrier crop.

Figure 6 above shows a well maintained field with corn as barrier crop around the perimeter. The field was without weeds at all times (except once when there was prolonged rainfall) as cultural control is a vital component of the Integrated Pest Management (IPM) programme. This aims to make the environment less favourable for pests by removing alternate hosts as well as providing adequate sunlight and aeration. As the sweet potato vines grew to have sufficient foliage, the need for manual weeding diminished as the weeds were outcompeted by the sweet potato for sunlight, space and food. Herbicides were applied to manage the grass and other weeds to approximately 10 feet around the plot as to eliminate a refuge for pests.

Results

Table 1; Results from pathology and nematology tests conducted on soil samples from the trial area.

Soil Sample Collection Date	Pathogens	Nematodes
July 10, 2017	Incomplete Analysis	*Scutellonema* sp. (Non-parasitic Nematode)
September 23, 2017	*Beauveria bassiana* present in B1T1 and B3T1. *Aspergillus* spp., *Drechslera* sp., *Curvularia* sp. *Cladosporium* sp. *Fusarium* sp. were also found. These organisms are all commonly found in the soil.	Incomplete Analysis
November 20, 2017	Incomplete Analysis	Incomplete Analysis

It would have been ideal for the results of the Pathology test to be available for the initial soil sampling to determine the presence or absence of the *Beauveria bassiana*. This entomopathogenic fungus may be found naturally occurring in soil. Antagonistic effects of the fungi *Aspergillus flavus,* and *A. parasiticus* (Eurotiales: Trichocomaceae) species on *Beauveria bassiana* has been reported (Toledo, Alippi, & de Remes Lenicov, 2011). To a more extreme extent, the author demonstrated that *Bacillus thuringiensis* (Bt) is also an antagonistic bacterium to the *B. bassiana.* The insecticide Agree with the active ingredient Bt. was recommended for use to manage the Southern armyworm and hornworms on November 4, 2017. It would therefore be no surprize if the results from the final soil sample indicated an overall absence of *B. bassiana* as the Bt. produces antifungal properties that parasitize mycelia and conidia of the entomopathogenic fungus.

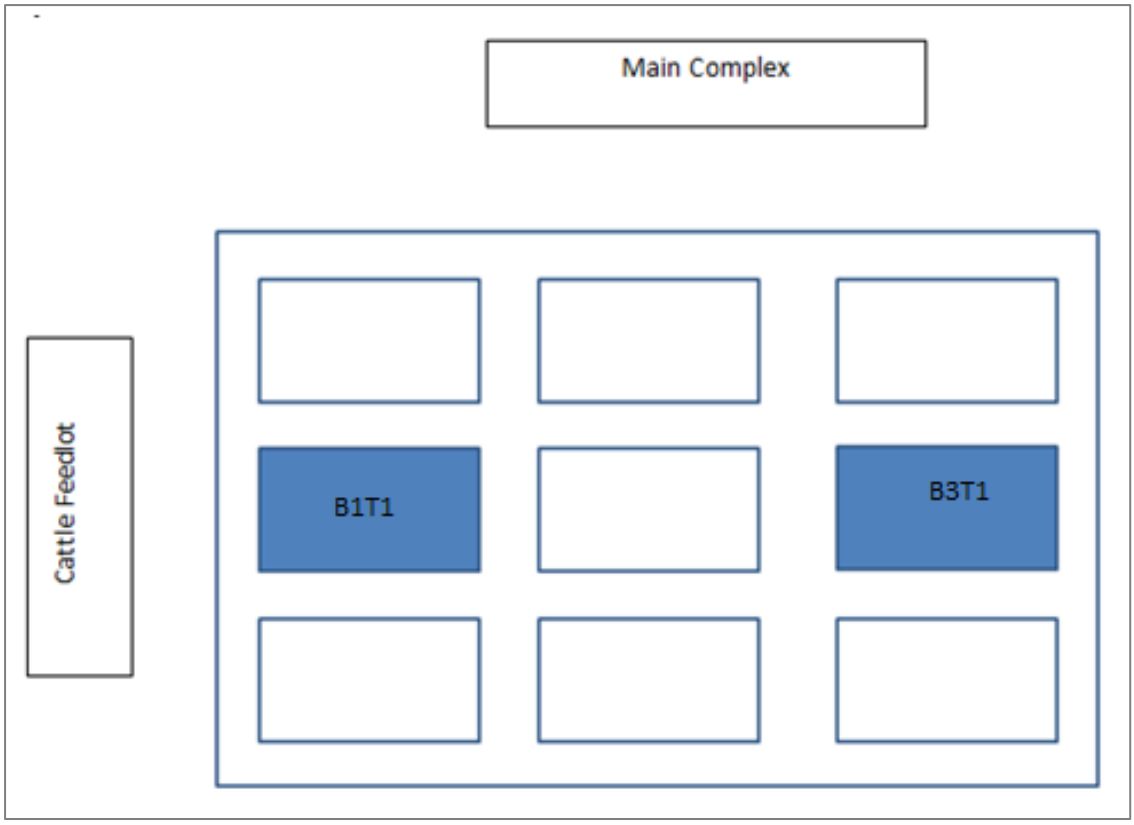

Figure 12; Visual representation of areas found present for the *B. bassiana* for the second soil sample done mid-way the cropping cycle.

Table 2; Mortality of the 2 varieties (Beauregard and Uplifter) of sweet potato slips used in the trial.

	August 21 (Initial planting date)		**August 29 (Supplied)**		**September 23 (Resupplied)**	
	Beauregard	Uplifter	Beauregard	Uplifter	Beauregard	Uplifter
Individual Varieties	360	360	220 (61%)	241 (67%)	116 (32%)	96 (27%)
Total (Beauregard + Uplifter)	720		461 (64%)		212 (29%)	

*( ) values expressed as a percentage of the corresponding value on the Initial planting date.

Seven days after planting, 64% of the total plant stand died and were replaced by the supplies planted behind each block. The Uplifter had a higher (67%) mortality rate than the Beauregard (61%) at this stage and the extra Uplifter slips had to be sourced from the diminishing germplasm. There slips were then treated in insecticidal and fungicidal dips prior to planting. On September 23, there was a lower mortality of overall plants as 29% of the plant stand died and was still considered a high death rate. At this point, there were more Beauregard (32%) dead than the Uplifter (27%). The stock behind the plot was now exhausted and the Beauregard was sourced from fresh material in the greenhouse (G1 slips) while all the Uplifter were taken from the germplasm in which good quality slips were hard to find. The slips were at different generations, different viral load and different quality of meristematic regions amongst others. It is now important that we get it right for the repeat trial and begin with ‘clean’ planting materials as the result is dependent on this step.

Figure 13; section of the field remained flooded after the rainfall.

Figure 13 illustrates water logging in Block 3 after heavy rainfall. These plants were stunted in growth when compared to those from blocks 1 and 2 which had better drainage that facilitated the movement of water away from those plots. Oxygen deficiency due to water logging may have caused sub-optimum nutrient supply as well as the synthesis of ATP may have been inhibited which would subsequently lead to a decrease in nutrient uptake (Steffens, Hutsch, Eschholz, Losak, & Schubert, 2005). The waterlogging and anoxic conditions not only affected the sweet potato plants in that area but also the barrier crop. The corn in that section of the field had stunted growth and the leaves had yellowing which were symptoms of waterlogging. Also the leaf area index was smaller for these plants than those in blocks 1 and 2 as well as there may possibly be a reduction in root numbers (Cannell, Belford, Blackwell, Govi, & Thompson, 1984) which would subsequently lead to a reduction in tuber yield. This depression in Block 3 was as a result of the initial field preparation as the beds were not raised as high as those in the other blocks. Small trenches were made to drain the excess water away from the block; however that was limited due to the presence of plants and drip lines. Care should be taken for the repeat trial to select an area that is uniformed (depth of soil and height of beds) and has mechanisms for adequate drainage.

The root system absorbs water and nutrients and also acts as the anchor of the plant. The sweet potato plant is made up of three root types; fibrous, pencil and storage roots (Masango, 2014). After the plant has ceased producing fibrous roots, it then channels its energy into bulking of storage roots.

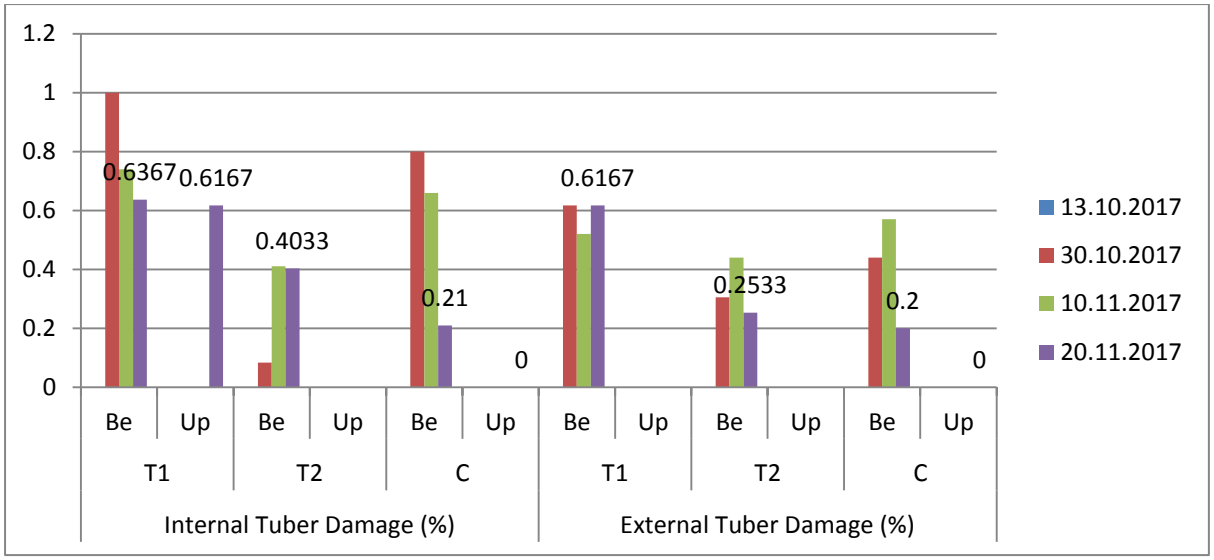

Figure 14; Percentage (%) internal and external tuber damage for Beauregard and Uplifter for four harvesting days.

Although Beauregard tubers were harvested at each harvesting day, there were no Uplifter tubers on October 13 for the Control blocks, none on October 30 for all treatments, none on November 11 for Treatment 1 and none on November 20 for Treatment 2. According to Figure 9, both internal and external damages to the tubers of both varieties remained below 1%. Overall, Treatment 2 had the least amount of damage (both internal and external) which was followed by the Control group while Treatment 1 had the greatest level of damage. It is very important to note that there were no stage (egg, larva, pupa, adult) of the Sweet Potato weevil seen during examination by destructive sampling. One unidentified pupa was seen and is currently undergoing identification in the Entomology laboratory at Bodles Research Station. There is sufficient evidence which suggest that other insect pest may have caused these damages. The sweet potato weevil has the tendency to feed deeper into the 'flesh' of the tubers but this insect only remained closer to the potato skin. There were not enough data available to do a detailed comparison of the effects of the *B. bassiana* on the SPW for the 2 cultivars of sweet potato as the Uplifter did not develop storage roots in most instances.

Figure 15; sweet potato weevil pheromone trap installed in the field.

The SPW pheromone trap was placed in the field on the 22nd of August 2017; one day after planting. Since installation of the trap until the end of the trial on November 20, 2017, there has been no SPW caught in the trap. This is due to the prolonged period of rainfall which successfully suppressed the weevil population to very low levels. There is a continuous SPW trapping across Bodles Research Station (North and South Bodles) which also indicated that the weevil population is very low as the last catch that has been recorded to date was in September when there was a two and a half weeks break from rainfall.

Due to the effects of climate change, it is expected that the pattern of rainfall (periodicity) will change in a way that most of the rainfall occurs in a particular period of the year (wet season) and the dry season is more extreme where we have very high temperatures and very little rainfall. The *B. bassiana* does not do well at high temperatures and high soil moisture content. Conidia half-lives ranged from 14 days at 25°C and 75% water saturation to 276 days at 10°C and 25% water saturation (Lingg & Donaldson, 1981).

Figure 16A; Cucumber beetle; ***16B;*** sweet potato hornworm; ***16C***; Southern armyworm.

All major damages that were inflicted upon the sweet potato crop were caused by foliage feeders. The cucumber beetle (*Diabrotica* sp.) in figure 16A was a major pest as they were seen in relatively high numbers since leave started to grow on the plants. They were initially accompanied by whiteflies after which the whiteflies population diminished. During this early stage of the crop, nutrients may have been lost which would have otherwise been used for growth and production of metabolites that would be translocated for the early development of storage roots. This loss in leaf surface area caused by the cucumber beetles resulted in lower photosynthesis, hence lower production of food.

The sweet potato hornworm (*Agrius sp.*) and the Southern armyworm (*Spodoptera eridania*) appeared later in the crop, however they consumed the foliage at a fast rate. This forced the team to apply a safe chemical, Agree, which would have little or no impact on the SPW.

References

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