
# IPM CRSP

## INTEGRATED PEST MANAGEMENT COLLABORATIVE RESEARCH SUPPORT PROGRAM

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## Impact

This study further confirmed that black weevil damage is very serious in plantain and that traps in use are not enough to monitor the damage caused. Management of plantain residues should be an important IPM component to reduce population increase of the insect.

## Networking Activities

Research results and experience on this crop has attracted attention of the extension service and farmers organization of the region. Regular contacts have been established with INIBAP with relation to plantain research and some technical support has been offered.

## Project Highlights

Although this trial was not designed to evaluate every component of IPM management on plantain, it is becoming clear that some of the practices include improve resistance of plants to disease and pest incidence. Clarifying some aspects of the main constraints (Black Sigatoka and Black Weevil) as being pursued with other activities, may make those practices even more efficient. For Black Sigatoka, it seems that fungicide application may be an important IPM component and more studies should be done with respect to doses, frequency and combination with epidemiological parameters. For Black Weevil, we need more information about the behavior of the insect in order to design proper ways to lower populations through control practices.

### References

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# II.5: Mass Production of Local Strains of Entomopathogenic Fungi to Control Black Weevil in plantain

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## Abstract

**From January to September, 2001 cultures of *Beauveria bassiana* were isolated from plantain plots of Pichilingue and El Carmen. In each place, type V traps were set at weekly intervals and all weevils present on four successive days were taken to Plant Pathology Lab at Pichilingue. Weevils were washed 3 minutes with commercial chlorine (5%) and rinsed with sterile distilled water before inoculating them on Potato-Dextrose-Agar and Agar-Sabouraud and then incubating at 24 °C. Cultures obtained are kept in tubes with PDA at 15 °C. Several substrates made of locally cultivated grains (rice, oat, corn meal, soybean and quinua) were tested for efficiency of colonization and sporulation of *B. bassiana*. The best result was obtained with rice soaked for two days before inoculation. Simultaneously, several plastic bags from local market were screened to choose one that tolerates autoclave sterilization. To test the efficacy of the fungus against weevils, weevils inoculated with an strain of *B.* bassiana (Pichilingue 1) were put into a humid chamber with pieces of pseudostems. At the fourth day the fungus colonized and killed 100 % of weevils. The *B. bassiana* isolate from Pichilingue seems to be highly aggressive.**

## Objectives:

1. To obtain local strains of entomopathogenic fungi from Black and Striped weevils of plantain mainly *Beauveria bassiana* & *Metarrhizium anisopliae*;
2. To validate, adapt and develop mass production methods for the above fungi; and
3. To establish and maintain collection of entomopathogenic microorganisms associated with plantain in Ecuador.

## IPM Constraints

It is estimated that around half of the 60% yield loss of plantain is due to Black Weevil attack; the debilitating effect that the larval stage of this insect cause in the corm of plantain is well documented in every country where plantain is grown. The best control currently available is use of traps

with insecticides, the latter with high environmental and personal risk. However, as has been demonstrated in other activities of this Project, where the pest is endemic as in the plantain belt in Ecuador, trapping has little or no effect on the insect population and damage remain similar throughout the year. The existence of entomopathogenic fungus is as well documented in former research reports, both from this same project and from others in different countries, mainly Colombia, Costa Rica and Brazil, where most of the plantain research has been developed. The existing control potential for the fungi *Beauveria bassiana* and *Metarhizium anisopliae* against this pest has been established. Its use has not been diffused to farmers because the available commercial formulations are expensive and not always effective. In addition, manipulation of this formulation has certain limitations through humid and hot climates as in the Ecuadorian region. It has been proposed that locally isolated strains may be more specific and efficient.

## Research Methods

**The following procedure was established from January to May 2001:**

Weevil collection. - "V" traps were set weekly in a plantain plantation with poor management practices within the Experimental Station in Pichilingue. Traps were set on Mondays and then every day till Friday, all the adult weevils present were collected in a Petri dish and taken to the lab. No insecticides were added. The plantation was showing a high weevil infestation and frequently some plants fall over due to root destruction. In these cases, galleries in the corm were explored and all the larvae present were collected.

Insect Processing. - Adults and larvae were initially cleaned with sterile distilled water (SDW), disinfected with a 5% solution of commercial Sodium hipochlorite during 3 minutes, and three rinses with SDW and placed in Petri dishes with a general agar media (Potato Dextrose Agar-PDA). Since no results were obtained with this procedure, which produce only bacteria or nothing, the specimens were placed in humid chamber after the cleaning and disinfection. Chambers were prepared with humid towel paper and pieces of corm. Corm was changed every three days. Daily observations allow detection of symptoms of infection on some of the insects and only those were then proceeding to the next step.

Fungal isolation. - Sporulated samples of adults and larva were inoculated in both PDA and Agar Sabouraud (Merck) amended with chloranfenicol (500 mg/l of media). Sub culturing was necessary to obtain pure colonies. These were then maintained in tubes with PDA at 15$^0$C for future use. It has been observed that the viability and strength of the spores are found in good conditions after 5 successive subcultures (approximately once every 3 weeks).

Substrate and container trial. - Different substrates made of grains, whole or mashed, with and without molasses added were screened to test capacity to sustain fungal growth and sporulation. Three Erlenmeyers flasks of 150 ml were used for each of the following media: whole grains of commercial rice, quinoa, oat and soybean, 10 g each mix with 20 ml of distilled water (DW); rice, 10 g with 16 ml of DW; rolled oat + 10 ml of DA; 20 g of corn meal + 30 ml of DW; soybean meal, 10 g + 20 ml DW; and 10 g of rice kept in water for 2 days. All media were autoclave sterilized once for 20 minutes, except the oat that had to be sterilized twice. One colony of *B. bassiana* (named Pichilingue 1) was used to inoculate this entire media.

To evaluate the growth of the fungi, a visual scale of 5 points was used to qualify the degree of mycelium covering the medium and the corresponding sporulation. (0 = no growth/sporulation and 100 = complete covering of the media). The best container for distribution to local farmers should be cheap, easily available and safe for the fungus to be handled. Therefore, the first thing tried was a range of plastic bags available in the local market. They were independently placed in the autoclave and sterilized for 20 *minutes to establish their resistance.* Fortunately, the first round gave one type of bag that was resistant to this procedure and was selected for the rest of the trial.

Fifty grams of the media selected was placed on each bag, this was sealed with staples and then sterilized for 20 min. Seven-day-old cultures of *B. bassiana* were used to prepare a suspension of spores (10 g of colonized grain + 100 ml of SDW, filtered through cheesecloth). Five ml of the suspension were injected in every bag; the point of injection was sealed with adhesive tape. Inoculated bags were incubated at 4$^0$C for 7 days. Then they were kept at ambient temperature in the lab for several weeks to test its viability and "shelf life".

Pathogenicity test. - Black (11) and Striped (30) weevils were collected from infested plants from different plots from the Experimental Station. They were washed with SDW and then placed within a big plastic bag with a piece of plantain pseudostem cut by the middle like a trap, and spread with some of the rice grains with a 25 days old colony of the fungus. One side of the bag was replaced with a nylon mesh to allow oxygen exchange.

## Research Results and discussion

**Fungal isolation**

The field traps render around 15 to 20 weevils daily with a rate of 1:3 Black to Striped, respectively. Around 15 larvae were collected weekly. From around 1300 adults and 300 larvae observed, only 2 cultures were recovered from Pichilingue fields and are kept in agar media (PDA). One of these cultures was used for subsequent tests. Similar collection started in El Carmen. Adult insects were

collected once a week and taken to Pichilingue for processing. In this case, results were more encouraging and up to now, 16 cultures have been isolated. Apparently we may conclude that conditions for natural biocontrol are more favorable in El Carmen than in Pichilingue. Information obtained during years 5 and 6 points at 24% parasitism of *C. sordidus* by *B. bassiana* en El Carmen.

Substrate and container trial. - Table 1 shows levels of mycelia growth and sporulation on the substrates tested. It was evident that rice was the best substrate for the fungus and among rice, the best one was soaked rice.

**Table 1. Percentage of growth and sporulation of *B. bassiana* on different substrate. Average of three repetitions. Pichilingue, 2001. INIAP-IPM/CRSP.**

| Substrate | Growth % | Sporulation % |
|---|---|---|
| Corn meal | 80 | 20 |
| Oat | 50 | 80 |
| Rolled oat | 75 | 10 |
| Quinoa | 80 | 50 |
| Rice + 20ml DW | 100 | 100 |
| Rice + 16ml DW | 100 | 100 |
| Rice, soaked 2 days | 100 | 100 |
| Soybean | 40 | 5 |
| Soybean meal | 45 | 5 |

The same kind of growth was obtained on the plastic bags with soaked rice. Five days was enough to obtain 100 % esporulating media. The bags maintained in the lab were still in perfect conditions 25 days afterwards. In order to check the amount of spores produced, samples were diluted and counted with a hemocitómetro, resulting in 145 x $10^6$ spores per gram of colonized media.

## Impact

The possibility of local mass production of entomopathogenic fungi may facilitate further work on biocontrol of this pest.

## Project Highlights

The media and container for distribution of the entomopathogenic fungi *Beauveria bassiana* under Ecuadorian conditions is ready for testing at the field level in order to obtain a more efficient biocontrol of weevils in plantain.

The procedure to recover local colonies as is in use at the Pichilingue Experimental Station lab and it is expected that in the near future, local strains from the main plantain area will be collected.

# II. 6. Effects of Trapping systems and other IPM Practices on the Population Dynamics of *Cosmopolites sordidus* on Plantain in Ecuador

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C. Suárez-Capello (INIAP-EET Pichilingue); I. Carranza (Owner of the farm where experiments were performed)

## Abstract

**As a first step to establish appropriate control measures against Black Weevil within an IPM system for plantain, an experiment was set up to test the effectiveness of different types of traps for *Cosmopolites sordidus*. The experiment was located at a 15 year-old farm in the El Carmen area. Treatment were five types of traps , made with pseudostems and corms of plantain. Weevils were collected 24 and 72 hours afterwards. Traps type A (sandwich over a corm) and C (lateral cut) caught the largest quantity of weevils: 5 to 7 per trap in average against 1 to 2,5 on the other three.**

## Objectives

To determine the effectiveness of different types of traps for *C. sordidus*;

Evaluate natural and synthetic attractants as indicators of relative abundance of weevils; and
Establish an appropriate methodology to improve biological and cultural control practices for black weevil in plantain plantations.

## IPM Constraints Addressed

A literature survey on Black weevil (*C. sordidus*) control measures established the use of traps with some kind of insecticide as a means of reducing population. The increasing demand of plantain for export, prompt farmers to use highly dangerous insecticides as Furadan (Carbofuran) and Lorsban (Clorpiriphos) to keep *C. sordidus* under control. Although there are no exact figures, it is estimated that more than 30% of yield is lost due to this pest.