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Integration of pheromones and the entomopathogenic fungus for the management of the banana weevil

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Abstract

Candidate strains of the fungus *Beauveria bassiana* have been identified for use in the integrated pest management of the banana weevil (*Cosmopolites sordidus*). However, the important limiting factor has been the lack of an economic and effective delivery system to maximize field effects. Catches in pheromone traps are low and, currently, costs of using *B. bassiana* are high. The two methods of pheromone trapping and application of fungus, may be integrated to provide a cost effective strategy for the control of the pest. We conducted studies to determine the potential for the pheromone-baited traps to aggregate the banana weevil around the trap mat and to determine the effect of different delivery systems of *B. bassiana* using pheromones. Our data show that twice as many weevils were captured in pseudostem traps at the base of the trap mat (pheromone-baited mat) than at mats < 5 m from the trap mat, and four times as many than at mats > 5 m away. The results suggest that many weevils are attracted to pheromone lures but fail to enter the traps, resulting in weevil aggregation in the vicinity of pheromone traps. We observed that infected weevils can transmit the fungal pathogen to healthy individuals. A high percentage of weevils died due to *B. bassiana* infection in plots where *B. bassiana* was applied on the trap mat and four adjacent mats (14.2%) than in plots where the pathogen was applied in the pheromone trap (4.1%) or around the pheromone trap (7.2%). An on-station study to determine the effect of integrating pheromones and *B. bassiana* on weevil population size and weevil damage is being conducted.

Keywords: *Beauveria bassiana*, delivery system, Musa spp. , transmission, pheromones

Introduction

The banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important insect pest of bananas (*Musa* spp, AAA-EA genome group) and plantains (AAB) in Africa (Gold *et al*., 2001). Weevil oviposition takes place at the base of the plant. On hatching, the larvae bore in the corm which results in a reduced nutrient uptake of the plant. Attack in newly established banana stands may lead to crop failure. Heavy infestation in established fields may result in reduced bunch weights, mat disappearance and shortened life span of the plants (Gold *et al*., 2004a).

Adult banana weevils are soil dwelling nocturnal insects that are rarely observed in banana stands (Gold *et al*., 2001). They are often observed in the base of the leaf sheaths or in and around the corm (Gold *et al*., 2004b). Weevil movement has been reported variously to range from 6 m in one night to 60 m in 5 months. In an experiment conducted at Kawanda near Kampala, Uganda, 42% of recaptured weevils were found within 5m of the point of release and 39% had moved 5-15m and only 3% had moved more than 25m six months after release. Females tended to be more active than males. A small proportion of weevils were observed to remain on their release mat after 2 weeks of release (Gold *et al*., 2001). Control of the banana weevil has been difficult. Use of entomopathogens provides a plausible method for the management of this pest. Candidate strains of *Beauveria bassiana* have been identified for use in a banana weevil integrated pest management strategy (Nankinga, 1999). An important limiting factor has been the lack of an economic and effective delivery system to maximize field effects. The potential of using pheromones for the delivery of fungal pathogens for the control of the banana weevil has been previously suggested (Budenberg *et al*., 1993; Gold *et al*., 2001; Tinzaara *et al*., 2002) but no research has been done to investigate whether this is cost effective. The use of pheromones as lures for dissemination of *B. bassiana* has been reported to be effective for other beetles (Vega *et al*., 1995; Klein and Lacey, 1999; Vega *et al*., 2000). In our case, the pathogen would be placed at the mats where the traps are positioned and the attracted weevils are expected to be contaminated with the pathogen and disseminate it to other individuals. However, the mode of application (e.g., around the trap mat or inside pheromone trap) of the pathogen will

need further investigation to optimise the delivery system of *B. bassiana* for *C. sordidus* control.Entomopathogenic fungi such as *B. bassiana* have the potential to grow, multiply and persist on the insect they kill. In addition, infected individuals can move away from the infected point, thus carrying the pathogen throughout the pest's habitat (Ferron, 1981). This may result in an increase of inoculum in the pest population possibly leading to an epizootic situation (Ferron, 1981). Infection of insects by the pathogen takes place in two phases. In the first phase, the pathogen germinates on the cuticle and enters the insect. The pathogen grows inside the host, forming budding spores (blastospores). In the second phase, after death, conidia are formed externally on the insects' surface. When healthy insects come in contact with the cadaver, they may be infected by the conidia. The insect may also transmit conidia to healthy individuals by contact before the conidia germinate and enter the insect. The fungus on the dead insect is reported to be more persistent than that on the substrate.

Ignoffo (1978) and Furlong *et al* (1995) urged the exploration and targeting pest species with entomopathogens and described the method of using the insects themselves to introduce and spread pathogens to the insect populations. In laboratory bioassays, Schoeman and Schoeman (1999) observed that *B. bassiana* could be transmitted from infected weevils to healthy individuals. Studies conducted by Godonou *et al* (2000) showed a possible dissemination of *B. bassiana* conidia from infected to non-infected weevils. Detailed information on how *B. bassiana* can be transmitted from infested individuals to non-infested ones will be important in developing an effective delivery system for the pathogen.

The objectives of this study were: (1) to determine the potential for the pheromone baited traps to aggregate banana weevils around the trap mat; (2) to evaluate field transmission of *B. bassiana* from infected to uninfected banana weevils and (3) to evaluate the different delivery systems of *B. bassiana* using pheromones for the control of the weevil.

Materials and methods

Site description

The studies were conducted at IITA's Sendusu Farm (00.32N 32.32E 1260 meters above sea level (m.a.s.l.), located 28 km northeast of Kampala (Uganda) and Kawanda Agricultural Research Institute (KARI) (0°42'N, 32°53'E, 1220 m.a.s.l.) located 12 km north of Kampala. Both sites have two rainy seasons (March-May and September-November) with mean annual rainfall of 1200-1250 mm and daily mean temperature of 21 °C.

Pheromones.

The pheromone lures for use in these experiments were obtained from ChemTica International. They were sent by courier, sealed in plastic bags with transit taking less than one week and subsequently stored in a freezer on arrival until use. Each pheromone pack contained 90 mg of Cosmolure+ releasing at a rate of 3 mg/day (A.C. Oehlschlager, pers. comm.).

The potential for the pheromone- baited traps to aggregate C. sordidus around the trap mat.

The experiments to determine whether or not the banana weevil may be attracted to and aggregate near pheromone-baited traps without entering the pitfall traps were conducted at Sendusu and Kawanda Agricultural Research Institute (KARI). The experiment at Sendusu was conducted in 8-year old banana plots (25 x 25 m, separated by 10 m alleys) planted with cultivar Atwalira (*Musa* spp, AAA-EA type). The plots were feed free by regular hand weeding, and were not mulched, and there were an average of 40 plants per plot. At the onset of the experiments, a mean of 2.1 adults weevils was captured per pseudostem trap from the plots. The weevils captured in pseudostem traps were released on the mat of capture after counting and recording their numbers.

The on-station field at Kawanda had been planted with the highland cultivar Atwalira (*Musa* spp., AAA-EA group). The field consisted of experimental plots (36 mats, 3 x 3 m arrangement) in which the weevil population had built up over the three years of the plantation age. A 5 m grass alley separated plots. The plots were well managed and mulched with elephant grass (*Pennisetum purpureum*). Prior to the placement of pheromone-baited pitfall traps, pseudostem traps (2/mat) were placed in the field for three days to give an indication of banana weevil abundance. The banana weevils captured in pseudostem traps were released on the mat of capture after counting and recording their numbers. Trap captures averaged 2 weevils in the Sendusu stands and 6 weevils per three days in the KARI plots.

A total of 12 pheromone-baited pitfall traps were left in each of the fields for 30 days. After the pheromone-trapping period, three pseudostem traps (Mitchell, 1978) were then placed at the base of each mat and all mats had traps placed on them. Data were collected on the number of weevils found in each pseudostem trap after three days and at three different distances from the pheromone lure, i.e., 0 m (trap mat), < 5 m and > 5 m. The data were square root transformed and analyzed using ANOVA of SAS (SAS® Institute Inc., 1999), and means were separated using the Student-Newman-Keuls test (SNK).

Field transmission of B. bassiana from infected to uninfected banana weevil adults.

This study was conducted at Sendusu to determine field transmission of *B. bassiana* from infected weevils to non-infected weevils, and to determine the locations in the field where infected weevils are found. The first trial was conducted in a plot planted with cultivar Nabusa (*Musa* spp, AAA-EA group). The second trial was conducted in another plot 200 m from the plot used for first trial. The plot size for both trials consisted of 7 x 6 mats, at a spacing of 3 x 3 m. The plots were unmulched and well weeded at the start of the experiments.

Crushed maize formulation of *B. bassiana*, provided by the pathology laboratory at KARI was used. To infect weevils in the laboratory, *B. bassiana* was topically inoculated to marked weevils. Healthy weevils were placed in 3 cm diameter petri dishes containing 2 g of maize-formulated *B. bassiana* (approx. 3×10^9 conidia/g) for 6 h. After 6 hours, infected weevils were placed in petri dishes (9 cm diameter) with moist tissue paper in the laboratory (25-27°C, 80-90 % r.h.) for 3 days before releasing them in the field.

Pseudostem trapping before release of weevils was conducted to determine if *B. bassiana* was present in plots. Two pseudostem pieces were placed per mat and checked after three days. The captured weevils (at least 3 per mat) were placed in petri dishes and taken to the laboratory for incubation and assessing of mycosis (% mortality). Weevils were placed in a petri dish with moist tissue paper at 25-27°C and 80-90% r.h. Observations for mycosis were taken every 3 days for 21 days.

Weevils were collected from Masaka, marked according to sex, and whether they were to be inoculated with the fungus (infected weevils) or not (uninfected weevils) and mat of release. Sixteen uninfected weevils (8 males: 8 males) were released per mat. After one week of releasing uninfected weevils, 10 infected weevils (5 males: 5 females) were released per mat in the same plot. Weevils were released in the plots (on marked mats) at dawn (7.00-8.00 pm) when they are active and can avoid predation.

In both trials, pseudostem trapping (one pseudostem piece per mat) was conducted after 7, 14, 21, 35 and 42 days of releasing infected weevils. Two pseudostem pieces were placed per mat and inspected after three days. For every sampling occasion, weevils captured were placed in vials according to mat of capture and whether released infected or uninfected, and taken to the laboratory for incubation.

At the time of checking pseudostem traps, sampling by searching to determine locations of weevils infected with the pathogen was conducted. Searching was conducted in the following locations: (i) trash, (ii) residues (iii) soil by mat, and (iv) plant base in leaf sheath. Weevils recaptured were recorded and those that had died without mycosis or those found alive were placed in petri dishes and taken to the laboratory for incubation. In the laboratory at KARI, weevils were placed according to recapture mat in petri dishes (9 cm diameter) lined with moist filter paper. The proportion of weevils infected with mycosis (% mortality) from each plot was recorded after 3 days and was run for 21 days for each sampling date.

Phermone-baited delivery systems of B. bassiana for the control of the banana weevil

The study to evaluate the rate of transmission of *B. bassiana* to the banana weevil using different systems was conducted in the banana field at Sendusu. Fifteen plots were selected and those that lacked some mats were re-planted. The plots consisted of 5 by 5 mats of cultivar Atwalira (*Musa* spp, AAA-EA group), spaced at approximately 3 x 3m. The treatments (delivery system) were: (i) control (nothing), (ii) pathogen only applied to central mat, (iii) pheromone + pathogen inside trap, (iv) pheromone + pathogen around the trap mat, and (v) pheromone + pathogen on trap mat and four mats less 5 m. The crushed maize formulated pathogen (200 g) was applied by spreading at the base of the mat in all cases except when it was placed inside a gallon trap. Each treatment was replicated three times. The experiment was repeated using the same plots six months later.

In both experiments, weevils collected from Masaka and kept in the laboratory for a week were used. Ten weevils (5 females and 5 males) marked according to their sex and mat of release were released at each of the mats. Weevils were released at dawn (7.00-8.00 pm) when they are active and can escape predation. There were marked according to release mat and sex.

The gallon trap with a ramp that allows both easy entry and exit of the weevils was used (Fig. 1). The trap was made out of a 5-litre jerrycan. A window was cut in each side of the jerrycan and the flap folded down to make a walk-in ramp.The gallon trap with a ramp was placed at the centre of selected plots. The pheromone-baited trap was not placed in plots for control and *Beauveria*-only treatments. The traps were placed two days after weevil release. The pathogen (crushed maize formulation, 200 g per mat) was applied according to the treatments above.

Pseudostem trapping was conducted after 14, 28 and 42 days. The weevils that were recaptured were observed for pathogen infection, recorded according to distance from the fungus source or pheromone-baited trap. The weevils were then placed in vials according to distance of recapture (trap mat – 0m, less than 5m, and more than 5m) and taken to the laboratory for inoculation and then assessing for mycosis (whitish fungal mycelial growth). In the laboratory, weevils were placed in petri dishes lined with a moist tissue paper according to recapture distance. The number of weevils infected with mycosis (% mortality) from each plot or treatment at different distances was recorded after every three days for 21 days. Each treatment was replicated three times.During the date of checking pheromone traps, inspection for dead weevils was done from the following

locations in the field: (i) trash, (ii) corm or pseudostem residues, (iii) soil by mat and (iv) plant base-leaf sheath. Weevils with mycosis were recorded and those dead without mycosis were taken to the laboratory for incubation.The pheromone lure was replaced when the pheromone lure sachets were observed to be empty. Data on percentage mortality of weevils was arcsin transformed and analysed using the GLM procedure of SAS (SAS® Institute Inc., 1990), and means were separated using Fisher's least significant difference (LSD). The $\chi 2$-test was used to analyse the association between weevil sex and the different locations where dead weevils were found.

Results

The potential for the pheromone- baited traps to aggregate C. sordidus around the trap mat

Twice as many weevils were captured in pseudostem traps at the base of the trap mat (pheromone-baited mat) than at mats < 5 m from the trap mat, and four times as many than at mats > 5 m away (Figure 2). Both experiments at Sendusu and KARI showed similar trends of the number of weevils captured in pseudostem traps at the different distances from the pheromone-baited trap.

Field transmission of Beauveria bassiana from infected to uninfected adults of the banana weevil.

None of the 120 weevils that were sampled in the field before the experiment showed any signs of infection after incubation for 21 days in the laboratory. Of the weevils that were recovered by searching in the first trial, 10.8 % and 6.7% of the weevils that were released uninfected and unmarked respectively were found dead due to pathogen infection (Table 1). In the second trial, 8.2% of the weevils that were released as uninfected individuals and 4.2% of those that were recovered as unmarked weevils were found dead due to pathogen infection. Of the weevils, which were recovered by pseudostem traps in the first trial, 4.5% of the weevils released uninfected and 2.0% unmarked weevils died due to pathogen infection after incubation in the laboratory for 21 days (Table 2). In the second trial, 7.8% of the weevils released uninfected and 5.9% of the unmarked weevils died due to pathogen infection after incubation in the laboratory.

Our observation on the locations preferred by dead weevils infected with the pathogen indicated that most of the weevils (>72%) that were dead due to *B. bassiana* infection were recovered in the leaf sheath at plant base (49.6%) and soil by mat (23.5%) in the first trial (Table 3). In the second trial, more that 60% of the dead weevils due to pathogen infection were found in the leaf sheath at the base of the plant (54.7%) and soil by mat (10.7%). A small percentage of weevils were recovered from residues (13.4%) and trash (12.6%) in the first trial, while more dead weevils were recovered in residues (21.3%) than in soil close to the mat (10.7%) in the second trial. In the first trial, equal numbers of males and females were recovered within different locations in the banana field ($\chi 2$=2.86, df=3, P= 0.41). There were more females than males that were recovered at the base of the plant in the second trial ($\chi 2$ =10.02, df=3, P= 0.02).

Phermone-baited delivery systems of B. bassiana for the control of the banana weevil

In both trial 1 and 2, a high percentage of weevils were found infected with *B. bassiana* in plots where *B. bassiana* was applied in combination with the pheromone compared to where *B. bassiana* alone (Fig. 3). In trial 1, more weevils died due to *B. bassiana* infection in plots where *B. bassiana* was applied on the trap mat and four adjacent mats (14.2%) than where the pathogen was applied in the pheromone trap (4.1%) and around the pheromone trap (7.2%). In trial 2, the combination of the pheromone and the pathogen placed on trap mat and four adjacent also showed higher percentage of weevils that died due to pathogen infection (Fig. 3). In both trial 1 and 2, the delivery system of having the pathogen placed on trap mat and adjacent mats showed significantly (P< 0.05) higher percentages of dead weevils that were recovered at different distances from the trap than when *B. bassiana* was applied inside trap, around trap mat and when used independently (Figure 3). Of the weevils dead due to pathogen infection that were recovered by searching in trial 1 (Figure 4), significantly (P< 0.05) more weevils infected with the pathogen were recovered in plots where the pathogen was applied around the trap and four adjacent mats (55.6%) than where the pathogen was applied inside the trap (25.9%), applied around the trap mat (14.8%) and pathogen alone (3.7%). The percentage of dead weevils was statistically similar for traps where *B. bassiana* was placed inside the trap and around the trap mat (P<0.05). The results of searching for weevils in trial 2 showed a similar trend to that of trial 1 (Fig. 4).

Discussion

Successful use of pheromone lures in the dissemination of *B. bassiana* would require (1) that weevils can enter a trap, be exposed to the fungus and leave the trap; (2) that the fungus can be transmitted from infected to uninfected weevils to provide a greater effect than simple drowning of the weevils in a pitfall trap. In this study the potential of the pheromone to aggregate weevils around the pheromone-baited traps was demonstrated. The results suggest that many weevils may be attracted to pheromone lures but fail to enter the traps (only a small caught). This may result in weevil aggregation in the vicinity of pheromone traps. If so, it may be possible to integrate pheromone lures into a broader IPM program, e.g., by using pheromones to aggregate weevils at sites where the entomopathogen *B. bassiana* is delivered.

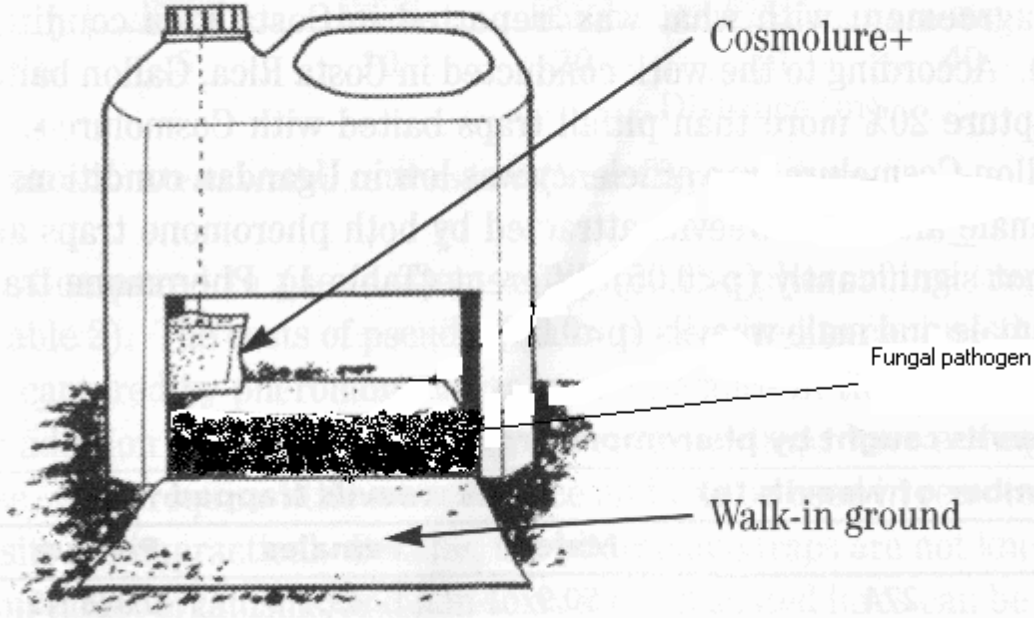

Figure 1: A gallon ramp trap that allows easy entry and exit of banana weevils

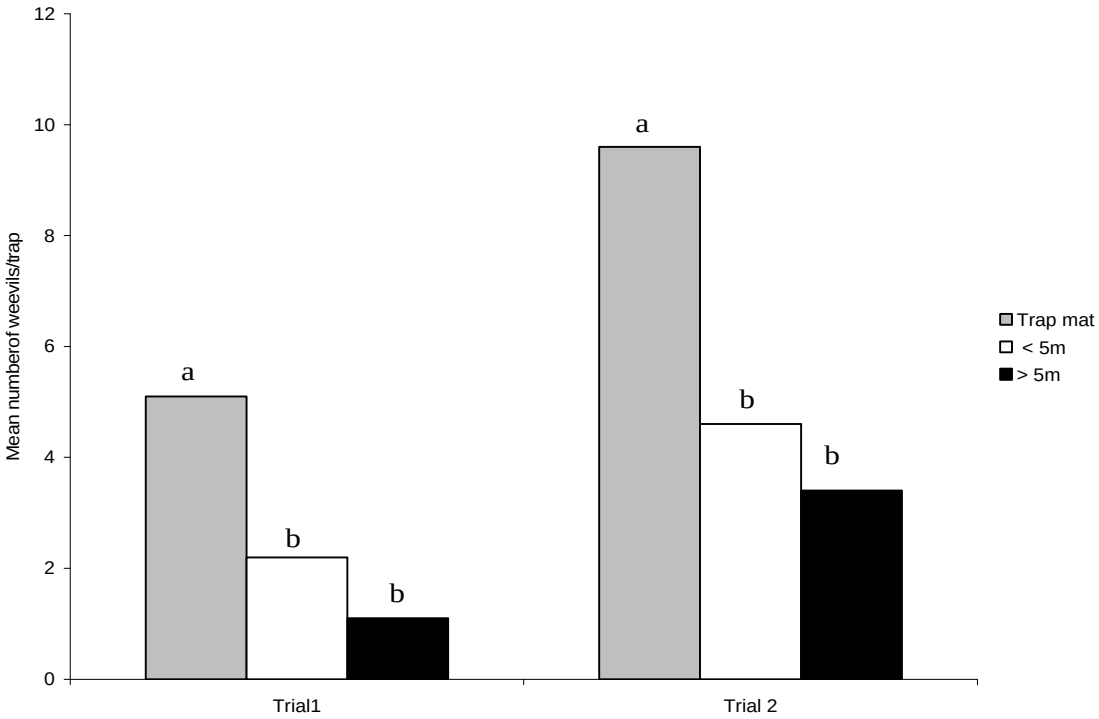

Figure 2. Mean number of weevils on mats at different distances from the pheromone baited trap. Means of bars with similar letters are not significantly different (P=0.05, Student-Newman-Keuls test (SNK)

Table 1. Percentage mortality due to *B. bassiana* infection of weevils recovered by searching that were released infected and non-infected in the field at Sendusu

Treatment	Number of weevils dead showing mycosis					
	Trial 1			Trial 2		
	Initial	Number dead	% Mortality*	Initial	Number dead	% Mortality*
Released infected	94	94	100.0	19	13	68.3
Released uninfected	74	8	10.8	73	6	8.2
Unmarked	104	7	6.7	95	4	4.2

* pooled data of three searching occasions per trial

Table 2: Percentage mortality due to *B. bassiana* infection of weevils recovered by pseudostem and incubated for 21 days

Treatment	Number of weevils dead showing mycosis					
	Trial 1			Trial 2		
	Initial	Number dead	% Mortality*	Initial	Number dead	% Mortality *
Released infected	12	9	75.0	35	18	51.4
Released uninfected	235	11	4.5	360	28	7.8
Unmarked	381	8	2.0	495	29	5.9

* Pooled data for five sampling occasions per trial

Table 3. Percentage of dead weevils due to *B. bassiana* infection that were recovered by searching in different locations in the banana field at sendusu

Trial	Location	Total number of recaptured weevils in each location			Mean % weevils with mycosis per location
		Males	Females	Total	
1	Plant base- leaf sheath	35	24	59	49.6a
	Soil by mat	16	12	28	23.5ab
	Residues	6	10	16	13.4b
	Trash	7	8	15	12.6b
2	Plant base- leaf sheath	13	28	41	**54.7a**
	Soil by mat	3	5	8	**10.7b**
	Residues	10	6	16	21.3b
	Trash	8	2	10	13.3b

Means with the same letter are not significantly different (Fisher's LSD, $P < 0.05$).

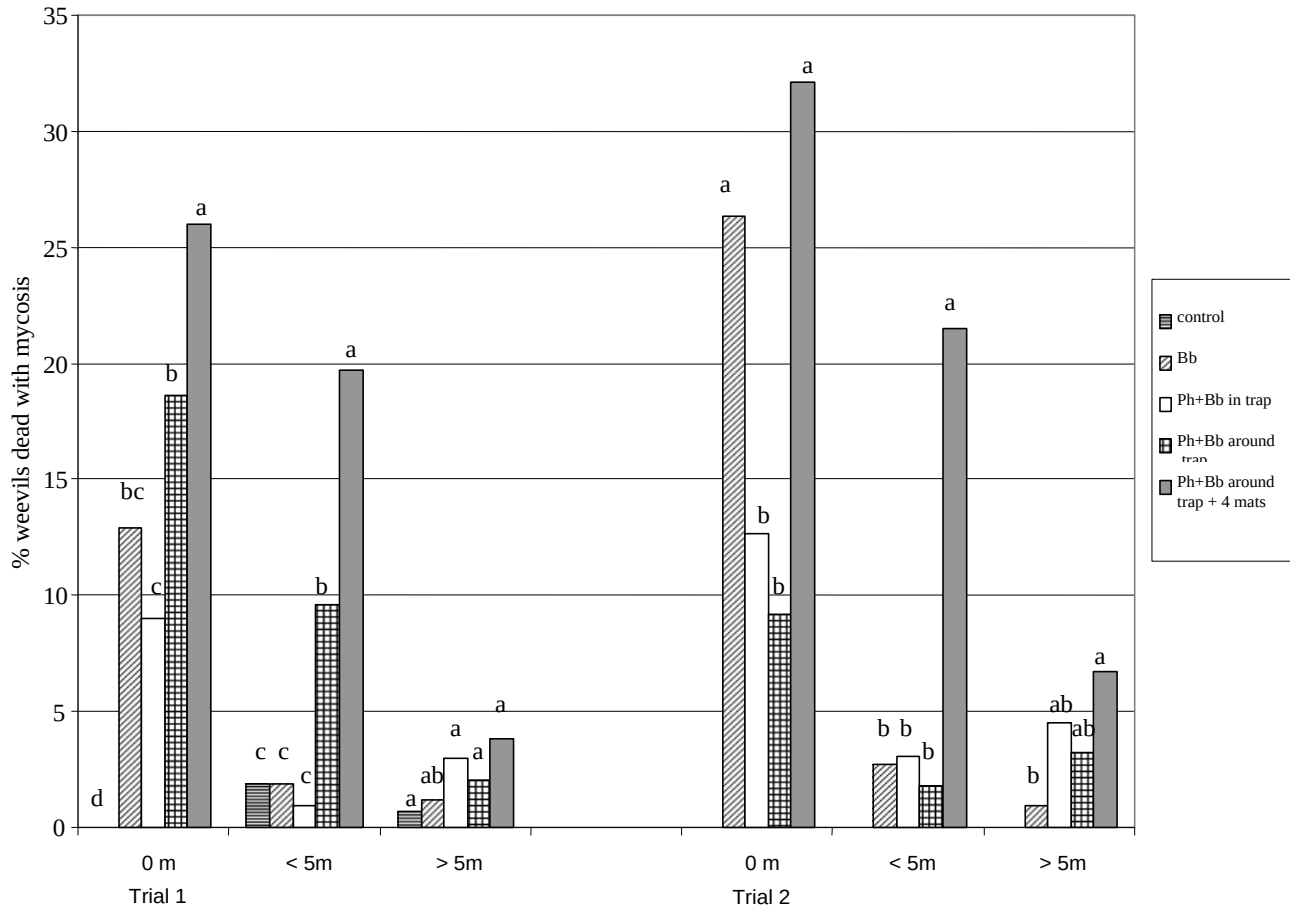

Figure 3. The percentage of weevils dead with mycosis after incubation for 21 days that was recaptured at different distances from the central/ pheromone trap mat of banana plots with different ways in which *B. bassiana* was applied. Bb=*Beauveria* (Bb) applied alone, Ph+ Bb in trap= *Beauveria* placed inside pheromone (Ph) trap, Ph+ Bb around trap=*Beauveria* placed around pheromone trap mat, and Ph +Bb around trap + 4 mats= Beauveria placed around trap mat and four adjacent mats. Bars followed by similar letters within a trial at each distance are not significantly different (P<0.05, Fisher's LSD),

The effective control of the banana weevil using pheromone in a delivery system for *B. bassiana* assumes that infected individuals can disperse from the infection point carrying the pathogen throughout the pest's habitat. It was observed in our study that weevils that were released uninfected were later found dead with the fungus and yet assessment of pathogen presence in plots was 0% before the trials started. The results indicate that uninfected and unmarked weevils that were found dead had been contaminated with the pathogen from the weevils that had been released infected. Our study demonstrates that the pathogen can be transmitted from an infected weevil to uninfected weevils in the field. Further investigation will be required to understand the factors that influence the transmission of the pathogen that will eventually lead to an epizootic.

Our observations on locations where the dead weevils infected with the pathogen go indicated that most cadavers were found in the leaf sheath at the base of the mat and in the soil near mat. The results have an important implication for the use of the pathogen for the control of *C. sordidus* as the weevils after death due to the fact *B. bassiana* infection go to locations where healthy individuals possibly go for oviposition and mating (Gold *et al.*, 2004a). This has the potential to increase chances of pathogen transmission during mating or individuals may be contaminated with the conidia from the cadaver. Detailed investigations into the behaviour of the weevil after infection with *B. bassiana* are currently being conducted.

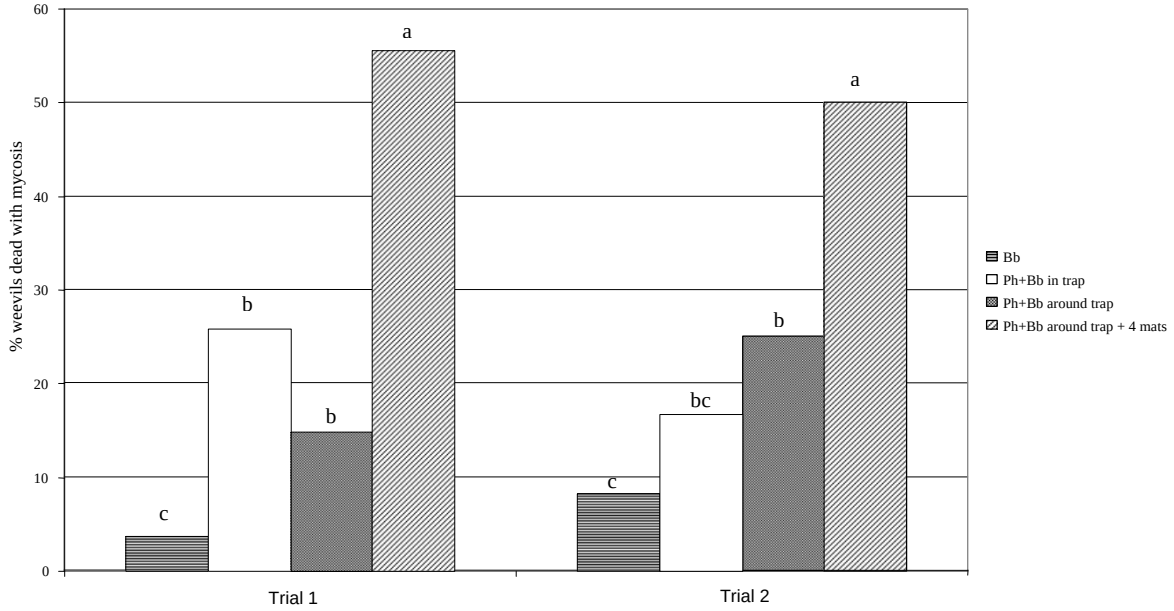

Figure 4. The percentage of weevils recaptured dead due to *B. bassiana* infection by searching at different distances from the pheromone trap or fungus release points in plots with different delivery systems. Bars followed by similar letters within a trial at each distance are not significantly different (P<0.05, Fisher's LSD

We know that the capacity to disperse, in addition to host factors, pathogen virulence, infectivity and persistence is a key factor in the ability of entomopathogens to develop epizootics (Roy and Pell, 2000). The use of an attractant in the system of introducing a deleterious agent into a pest population requires that the lured individuals can sufficiently disperse themselves after visiting the self-contaminating site (Klein and Lacey, 1999; Roy and Pell, 2000). In our study using different delivery systems, it was observed that a number of weevils that died due *B. bassiana* infection were recaptured at a distance of 10 m from the pathogen source. This suggests that these weevils were contaminated with the pathogen from the gallon trap baited with the pheromone and dispersed after infection. Placement of the pathogen into the pheromone trap mat and a few adjacent mats was found to be effective in delivering the pathogen compared to other delivery systems that were tested.

Our study demonstrated that weevils can aggregate around pheromone-baited traps and weevils can transfer the pathogen from infected to uninfected individuals. Studies are currently in progress to evaluate the effect of integrating pheromones with *B. bassiana* on weevil populations and damage. The data will be used in exploiting opportunities for integration of infochemicals with biological control. Further studies will be required to investigate the amount of pathogen that will be required to place in the pheromone trap for effective control of the pest. The longevity of the trap baited with the pheromone and the pathogen in one location before changing location before the delivery systems can be integrated in the management strategies of the banana weevil.

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