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Enhancing dissemination of *Beauveria bassiana* with host plant base incision trapfor the management of the banana weevil *Cosmopolites sordidus*

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The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important pest of highland banana in East and central Africa. It causes yield loss of up to 100% in heavily infested fields. Studies were carried out in Uganda to evaluate the efficacy of the the plant base incision trap in attracting C. *sordidus* and to determine the potential of using the trap in enhancing dissemination of the entomopathogenic fungi, *Beauveria bassiana* for the pest control. Field experiments were carried out in an established banana field of C. *sordidus* susceptible East African Highland Banana cultivar, Nabusa (AAA-EA). There were significantly more weevils recaptured in the incision trap (14.4%) than the conventional pseudostem trap (4.7%). After laboratory incubation, more weevils died due to pathogen infection from plots where the incision trap was used in combination with *B. bassiana* (25.9%) compared to where the pathogen was applied around the plant base without the incision (15.9%). Weevils showing signs of mycosis were recovered at 6 and 9 m from the pathogen release point, suggesting that the weevils can pick the pathogen from the aggregation point and disperse it. This data demonstrates that the incision trap is more attractive to *C. sordidus* than the conventional pseudostem trap and might be used to enhance dissemination of *B. bassiana* among adults.

Key words: Entomopathogenic fungi, horizontal transmission, pseudostem trap.

INTRODUCTION

The banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) is an important pest of highland banana in Uganda. The biology and pest status of *C. sordidus* have been reviewed by Gold et al. (2001)

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and Masanza (2003). The adults are most commonly associated with banana mats (a banana mat consists of plants coming from the common corm) and crop residues (Gold et al., 2001). They crawl short distances and may be sedentary for extended periods (Gold et al. 1999). Few disperse more than 50 m in three months (Gold et al., 2001, 2004). Eggs are deposited in the leaf sheaths and corm at the base of the banana mat (Abera et al., 2000). The larvae tunnel in the corm, damaging the vascular system and compromise the stability of the plant. Yield losses of 100% have been observed in farmers' fields (Sengooba, 1986), while losses of more than 40% have been reported in on-station trials (Rukazambuga et al., 1998; Gold et al., 2004).

Control of *Cosmopolites sordidus* is difficult because the immature stages (including the most destructive stage) are within the plant, often below the soil level, making them largely inaccessible to natural enemies (Gold et al., 2001). Therefore, control strategies often target adults. Currently, control options available to the farmers in Uganda include pesticides and cultural methods. Chemical control is regarded by farmers as easy to manage, fast acting and effective (Gold et al., 1993). The chemicals are however, costly and are thus not affordable by resource poor farmers in Uganda. Weevil resistance towards these chemicals has also been reported in some countries (Collins et al., 1991; Gold et al., 1999). Cultural control practices currently in use include crop sanitation and trapping using pseudostem but are of limited application due to being labour intensive and ineffective (Gold et al., 2002; Masanza, 2003). Farmer adoption of this method has also been limited by the availability of trapping material and lack of confidence of its effectiveness (Gold et al., 1993). Alternative control methods especially those that are ecologically sound, less expensive and safer to non-target species and environment than pesticides are being sought.

Bio-control using fungal microbes offers a promising means of managing this pest. For example, studies conducted in Uganda have identified eastern African strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo Vuillemin) (Ascomycota: Hypocreales) (Ugandan isolate G41) which effect > 95% weevil adult mortality in the laboratory and can significantly reduce adult population and damage under field conditions (Nankinga, 1999; Nankinga and Moore, 2000; Godonou et al., 2000). However, fungal microbes have not been used for the management of the pest by banana growers because of being expensive and not economically feasible.. This is because the weevil is a relatively sedentary insect, thereby necessitating the application of the fungus throughout the banana field. The possible use of infochemicals, especially kairomones, could provide a means of aggregating weevils at entomopathogen delivery sites in cuts made in residue corms, thereby reducing the amount of fungus and associated costs required (Tinzaara et al., 2002). The current delivery systems and their comparative advantage have not been investigated.

Cosmopolites sordidus is attracted to the kairomones from the host plant (Budenberg et al., 1993). The potential use of kairomones to aggregate banana weevils at delivery sites for entomopathogenic fungi has been reported (Tinzaara et al., 2002). The potential for kairomones to enhance transmission of *B. bassiana* needs to be demonstrated in Ugandan conditions. Treverrow and Bedding (1993) and Treverrow (1994) effectively used plant kairomones for the delivering of entomopathogenic nematodes by making conical cuts in the corms of banana stumps. Successful use of pseudostem traps or kairomones to enhance dissemination of *B. bassiana* would attract a large number of adults which have more chances to get in contact with the pathogen and infect healthy individuals outside the trap (horizontal transmission). This delivery system would have the advantage of reduced costs due to limited amount of the pathogen needed in traps and extra field application would not be necessary. Thus, the evaluation of techniques based on used of kairomones to enhance transmission of *B. bassiana* needs to be demonstrated in Ugandan conditions.

The objectives were to: (i) evaluate the efficacy of banana plant base incision trap for attracting *C. sordidus*, and (ii) determine the effectiveness of using host plant incision traps in enhancing the dissemination of *B. bassiana* in Ugandan conditions.

MATERIALS AND METHODS

Study site description

Laboratory and on-station field studies were conducted on the International Institute of Tropical Agriculture's (IITA) Sendusu Farm (0°32'N, 32°35'E, 1260 m.a.s.l) located 28 km northeast of Kampala, Uganda. The site has two rainy seasons (March-May and September-November) with mean annual rainfall of 1200 to 1300 mm and a mean daily temperature of 21°C.

Source of *B. bassiana*

Beauveria bassiana (isolate G41) (3×10^9 conidia/g) was obtained from the Pathology laboratory at Sendusu in a crushed maize formulation (Nankinga and Moore, 2000). This isolate had been previously shown to be effective against *C. sordidus* in the laboratory and field conditions (Nankinga and Moore, 2000).

Experiment 1: Efficacy of the incision traps

Experimental design

The objective of this experiment was to evaluate the effectiveness of using incision traps as compared to the conventional

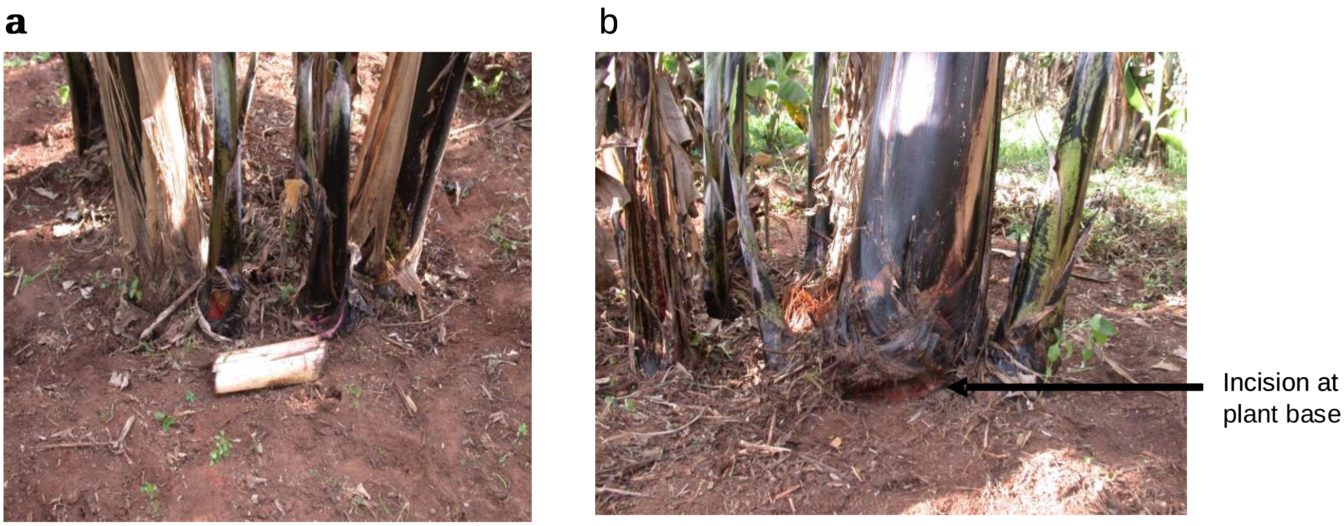

Figure 1. Trap types: (a) split pseudostem trap; (b) incision trap.

pseudostem traps in capturing *C. sordidus*. The experiment was conducted in five year old banana plots of East Africa highland banana, Nabusa cultivar (AAA-EA) mulched with dry banana leaves and well weeded. Plots of 7 rows by 7 columns of banana mats each (*Musa* spp., cv Nabusa, AAA-EA type) planted in a 3 m × 3 m arrangement were used. Plots were 10 m apart. The experiment comprised of two treatments: (a) Conventional pseudostem trap, and (b) Incision trap. Each treatment appeared in five randomly selected plots (that is, was replicated five times).

C. sordidus release and trap placement: Adult *C. sordidus* were collected from banana fields within the station at Sendusu using pseudostem traps (Mitchell, 1978). Weevil sex was determined using curvature of the last abdominal segment and punctuation on the rostrum (Longoria, 1968). Before release, weevils were scratched on the elytra with distinct marks for each banana mat per treatment using a surgical blade. A total of 72 weevils (36 female and 36 male) were released for each replicate on banana mats at varied distances (12 females: 12 males at 0, 3 and 6 m) from the traps for both treatments. Weevils were released in the evening (7.00-8.00 pm) at the base of the plants in each plot by placing them in shallow holes.

The split pseudostem traps were made from freshly harvested Nabusa cultivar plants obtained from the same field. The pseudostem was cut into pieces of about 30 cm long and split longitudinally to make uniform traps (Figure 1a). The traps were laid close to mats with their freshly cut surfaces in contact with the ground (Mitchell, 1978). The incision traps were made by making a horizontal cut/incision into the stump base at soil level using a strong sharp knife (Figure 1b). The incision was cut up to a half of the stump diameter and maintained slightly gapping by inserting small banana corm pieces (of about 2 cm^2 each).

Sampling and data collection

Weevils were picked from each trap after 3 and 6 days and identified using the marks to determine sex and the distance moved from the release point to the trap. The number of marked (males and females) and unmarked weevils recaptured in traps at 0, 3, 6 m from the point of release were recorded and the percentage of recaptured weevils calculated.

Experiment 2: Enhancing dissemination of *B. bassiana* using incision traps

Experimental design

The experiment was carried out in an established banana field at Sendusu and comprised of two treatments: (i) *B. bassiana* applied around the mat, and (ii) *B. bassiana* applied around the incision traps. Each treatment was replicated four times and each replicate was applied to separate plots of 7 by 7 plants (mats). Plots of 306.3 m^2 with 49 banana mats each (*Musa* spp., cv Nabusa, AAA-EA type) planted at spacing of 2.5 m × 2.5 m within rows were used. Plots were 5 m apart.

Application of B. bassiana

Two hundred grams (200 g) of *B. bassiana* maize substrate (5.9×10^7spores/g) was applied around one intact plant per plot for the treatment where *B. bassiana* was applied alone and around the base of incised banana stump for the incision treatment (Figure 2). The pathogen was applied on only one central mat per plot in both treatments. A treatment was applied to one plot. The plots where *B. bassiana* was applied were mulched using dry banana leaves obtained from the same field prior to treatments application (Figure 2). This was meant to prevent sunlight/heat effect on the pathogen.

Weevil release

The initial inoculum of *B. bassiana* in the banana plots was determined before release of weevils. Weevils were captured from plots using pseudostem traps and taken to the laboratory for incubation. Of the 100 weevils which were incubated for six days in the laboratory, none showed mycosis due to pathogen infection confirming no pathogen inoculum on weevils at the start of the experiment.

The weevils which were released in plots were first sexed and marked according to distances of release as described above. Twenty weevils (10 females: 10 males) were released on the mats at 0, 3, 6 and 9 m from the pathogen release point. Weevils were

Figure 2. *B. bassiana* applied around an incised plant base.

released in the evening (7.00-8.00 pm) at the base of the mats in each plot by placing them in shallow holes around the base.

Sampling and data collection

The weevils were captured weekly following their release and taken to the laboratory for incubation. The weevils were incubated (at 25 - 27°C, 80-90% r.h.) in separate glass petri dishes (90 mm diameter) in accordance to distance of capture from the *B. bassiana* release point (0, 3, 6 and 9 m). The incubated weevils were observed for any mycosis (whitish fungal mycelial growth) weekly for three weeks. The distance of infected weevil recovery from the nearest *B. bassiana* application point was noted.

Data analysis

Data of weevils re-captured from plots with pseudostem and incision traps was analysed using a chi squared test. The percentage of weevils recovered by pseudostem trapping from plots with different pathogen delivery systems that showed mycosis after incubation were analysed using contingency table test of statgraphics plus version 7 (StatPoint, Inc., USA) on numbers.

RESULTS AND DISCUSSION

Experiment 1: Efficacy of incision traps

The number of weevils recaptured from the incision trap was significantly higher (14.4%; n =360) compared to that from the conventional split pseudostem trap (4.7%) (t-test, P<0.05) (Figure 3). Males and females were attracted equally to either the incision trap or the pseudostem trap. There were significantly more weevils recaptured in incision traps compared to pseudostem traps from all distances from the trap (Figure 4). The incision trap attracted over 20% of the weevils from 3 m and beyond compared to 3% for pseudostem traps.

The banana weevil is attracted to the kairomones from the host plants (Budenberg et al., 1993). Treverrow (1994) identified iso-butyl-aldehyde and limonene to be the major components of the banana corm that are attractive to *C. sordidus*. In the current study, significantly more weevils were captured in the incision trap than the conventional pseudostem trap. This might be because of the higher concentration of attractive components such as limonene in the incision traps than in the conventional psuedostem traps (Treverrow, 1994). The results of this study demonstrate that incision traps are significantly more attractive to the *C. sordidus* compared to the conventional pseudostem trap.

The percentage of weevils captured from 3m and beyond was higher in incision traps than in pseudostem traps. The capacity for incision trap to attract large numbers of the weevils can be exploited for pest management where they are aggregated at delivery sites for entomopathogenic fungi for subsequent dissemination. If the incision trap attracts a higher proportion of the population to the pathogen delivery site, chances of transmission would be increased (Gold et al.,

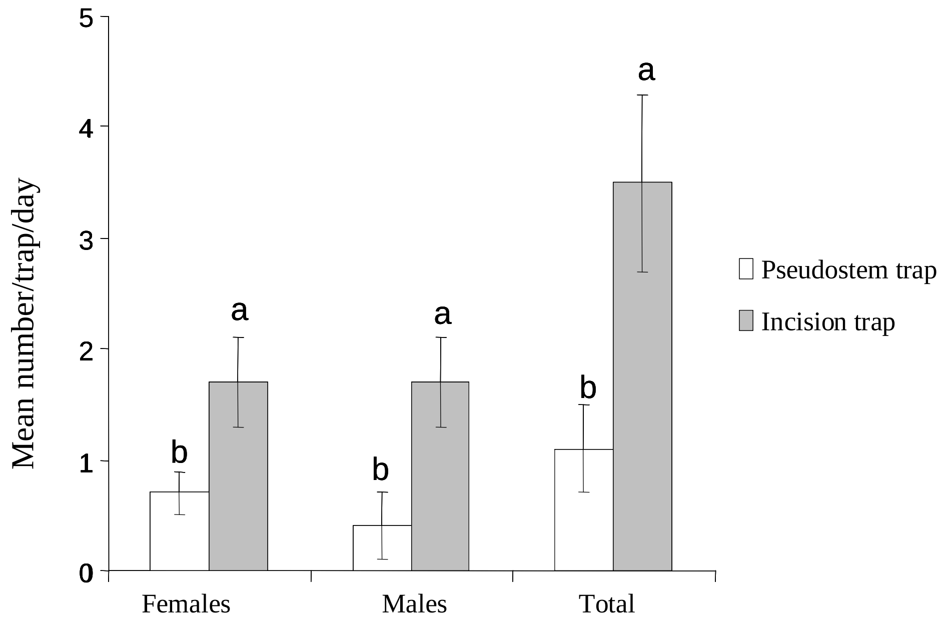

Figure 3. Mean number of *C. sordidus* captured in the incision trap compared with conventional pseudostem trap. Bars with different letters are significantly different, P < 0.05.

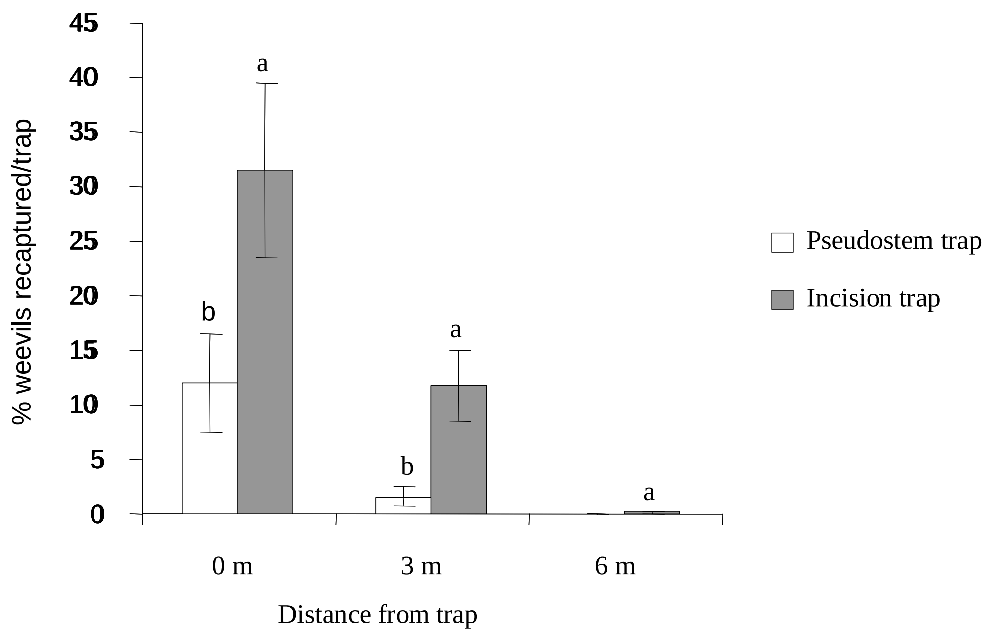

Figure 4. Percentage of *C. sordidus* recaptured in the incision trap and conventional pseudostem trap at different distance from the trap in the banana field at Sendusu, Uganda, 2006. Bars with different letters are significantly different (t-test, P < 0.05).

2001). Therefore, increasing trap efficacy will lead to higher transmission rates of *B. bassiana.* Theoretically, the strategy of using the kairomone to aggregate weevils on the pathogen delivery sites would have the advantage of reducing the amount of fungal pathogen applied per unit area compared to the technique of treating individual plants. Our results indicate that the incision trap can be used to attract *C. sordidus* to sites where the

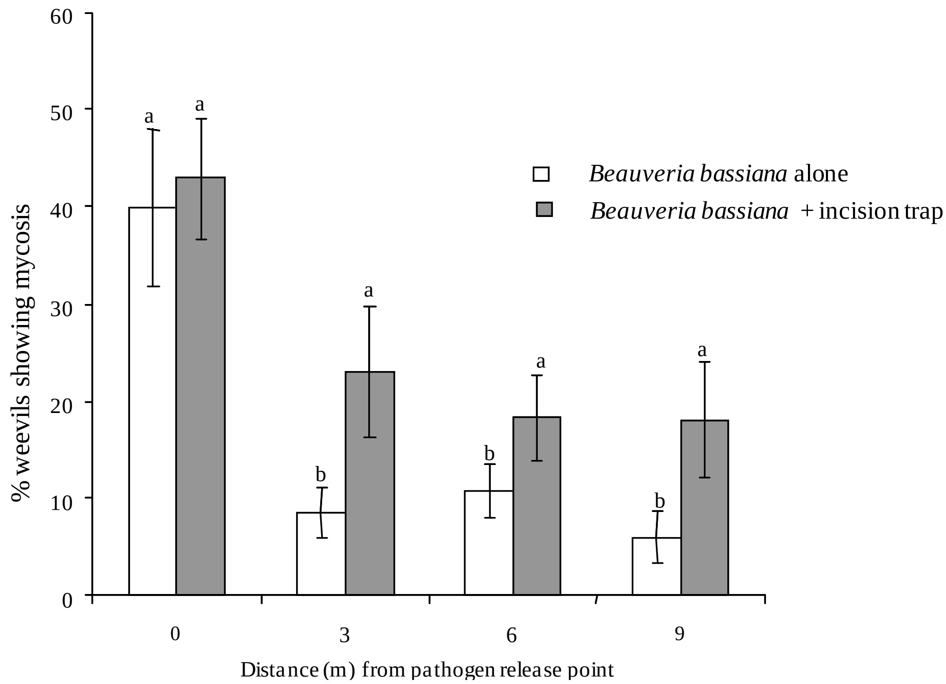

Figure 5. Percentage of weevils with mycosis that were recaptured at different distances from the pathogen release point in the banana field at Sendusu, Uganda, 2006. Bars with similar letters are not significantly different test, (χ^2; P <0.05).

entomopathogen *B. bassiana* is available for subsequent dissemination by the insects.

Experiment 2: Enhancing dissemination of *B. bassiana* using incision traps

Weevils recaptured from plots treated with *B. bassiana* around the incision trap showed significantly higher percentage mycosis (25.9±3.1) compared to those recaptured from plots where *B. bassiana* was applied around the banana mats without incision (15.9±2.7) ($\chi2$=19.2, d.f.=1, P=0.0001). The percentage mycosis for weevils recaptured at 0 m from the pathogen release point was similar among traps (Figure 5). Weevils showing signs of mycosis recaptured at 6 and 9 m from the pathogen release point was significantly higher where *B. bassiana* was applied around the incision trap than when it was applied alone. This observation suggests that the incision trap can enhance pathogen dissemination from the release points.

An effective mechanism of transmission is a key factor in the ability of entomopathogens to develop epizootics (Roy and Pell, 2000). The use of an attractant in the system as a method of introducing a deleterious agent into a pest population requires that the lured individuals can sufficiently disperse after visiting the self-contaminating site (Vega et al., 1995; Klein and Lacey, 1999; Roy and Pell, 2000). In our study using the incision trap delivery system, it was observed that a number of weevils that died due to *B. bassiana* infection were recaptured more than 6 m from the pathogen source. This suggests that these weevils were contaminated with the pathogen from the delivery sites and dispersed after infection.

In our study, 20 to 40% of the incubated dead weevils recaptured at different distance for the pathogen delivery point showed signs of mycosis. The result could have been overestimation as the incubation method and keeping weevils for a long time ensure infection that may not occur in the field. On the other hand, the percentage transmission seems to be low but it is possible that under suitable conditions (e.g greater soil moisture, higher *C. sordidus* population levels) such a level of infection would increase the overall inoculum level. The percentage transmission is also likely to increase when more pathogen is placed around the trap.

Conclusions

The results demonstrate incision traps are significantly more attractive to *C. sordidus* compared to the conventional pseudostem trap and it shows potential for spreading *B. bassiana* in the integrated management of *C. sordidus*. Further studies should be conducted to investigate the factors that can improve the percentage transmission of *B. bassiana* for the control of *C. sordidus.* There is also need to investigate the cost and benefit analysis of integrating the incision trap-*B. bassiana* delivery system in the management of *C. sordidus.*

Conflict of Interest

The authors have not declared any conflict of interest.

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