PROSPECTS OF BIOLOGICAL CONTROL OF THE COCOA MIRID, *SAHLBERGELLA SINGULARIS* Sahl (HETEROPTERA) IN GHANA: FIELD SURVEYS FOR ENTOMOPATHOGENS AND LABORATORY BIOASSAYS WITH *BEAUVERIA BASSIANA* ISOLATES.

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ABSTRACT

Distantiella theobroma (Dist) *is the most important species of mirids attacking cocoa in Ghana. Its control has mainly relied on the application of synthetic insecticides. Because of the hazards, cost and the resultant low adoption rate of research recommendations associated with such chemicals, the Cocoa Research Institute of Ghana continues to search for more cost effective and environmentally benign alternative means of managing the mirid menace in Ghana. Surveys were conducted for two mirid seasons; in all the six cocoa growing regions of Ghana for pathogens of S. singularis and species of Fusarium, Aspergillus, Nomuraea and Ascheinrsonia were isolated. Then followed four bioassays with five isolates of Beauvaria bassiana to assess their biological effect on S. singularis. The results indicated great potentials for the fungus as a biological control agent. Isolate I97 1035 was the most promising, followed by 197 1036, IMI 335249, I00 1183 and 197 1037. Details of the results are discussed.*

Keywords: *Cocoa, mirids,* Sahlbergella singularis, Beauvaria bassiana, *isolates.*

INTRODUCTION

Mirids (Capsids) (Heteroptera) are undoubtedly the most important insect pests of cocoa in Ghana and *Sahlbergella singularis* (Heteroptera) is considered the most prevalent of the four main species. For several decades, control of the bug and other cocoa mirids has mainly relied on the application of synthetic insecticides. Hazards associated with the use of such chemicals for pest control are known worldwide. In addition, chemical insecticides, applicators and other relevant inputs for their effective utilization have become increasingly costly for the average cocoa farmer in Ghana, resulting in low adoption rate of recommendations made by the Cocoa Research Institute of Ghana (CRIG) for chemical control of the pest (Padi 1991). CRIG, therefore, continues to search for more efficient, cost effective and environmentally benign alternative means of managing the mirid menace on Ghana's cocoa farms.

One possible alternative is the use of fungi that are pathogenic to insects, and such fungi are extremely important in microbial control of insect pests (Roberts and Humber 1981). Virtually all the insect orders are susceptible to fungal diseases, and this may be useful particularly for the control of sucking insects such as mirids (Roberts and Humber 1981). Among the fungi that have been exploited worldwide for insect pest control are *Metarhizium anisopliae* and *Beauvaria bassiana* (Ferron 1981), (Prior 1988), (Feng *et al.* 1994), (Oduor *et al.* 2000), (Godonou *et al.* 2000). Conidia of *B bassiana*, for instance, have been used to reduce populations of stored products pests of cereals by over 60 % by spraying onto the surface of bags in which these products were stored (Pham *et al.* 1995). (Adane *et al.* 1996) also reported that an isolate of *B bassiana* caused 88% mortality of *S zeamais* within 8 days and the pathogen was used successfully against the potato Colorado beetle, *Leptinotarsa decemlineata* in North America and Eastern Europe (Ferron 1978). In China, *B bassiana* has been used for the control of the European corn borer, *Ostrinia nubilalis* (Hussey and Tinsley 1981).

The humid nature of the cocoa ecosystem is believed to be conducive for rapid growth and sporulation of fungi, hence constituting a contributory factor to the menace of the cocoa black pod disease in Ghana (Dakwa 1973). It is possible, therefore, that such humid conditions would enhance the spread of fungal pathogens, such as

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B bassiana, of cocoa insect pests. *B bassiana* is also known to store well in a refrigerator at 5°C - 8°C without loss of viability and sporulation ability, whereas such temperatures will normally eliminate many other tropical fungi (Godonou 1999). (Lim *et al.* 1989) excluded *B bassiana* from the list of pathogens that are dangerous to humans and other mammals, but it has been cautioned that chronic exposure to high densities of fungal conidia could lead to allergic sensitization (Roberts and Humber 1981).

The use of *B bassiana* as a biological agent in the integrated control of mirids requires attention and this paper reports initial efforts at CRIG to include the fungus in the control of the pest, hopefully to minimize the use of synthetic insecticides.

MATERIALS AND METHODS

The project started with a search for pathogens of cocoa mirids in all the six cocoa growing regions of Ghana in August-January 2001/2002 and repeated a year later. Live, moribund and cadavers of cocoa mirids, as well as cadavers of other insects, were brushed into sterilized plastic vials and taken to the laboratory. They were incubated on moist filter paper placed in Petri dishes, observed for fungal growth and the causative fungi identified. Then followed four bioassays conducted with the following isolates of *B. bassiana* to determine their efficacies on *S singularis*: IMI 335249, I97 1035, I97 1036 and I97 1037 received from CABI Bio-Science in the UK, and I00 1183 originating from Ghana.

Experiment 1

All the five isolates were tested in the first bioassay. They were cultured on Potato Dextrose Agar (PDA) in 9cm diameter Petri dishes, and conidia extracted after 4-weeks with sterile distilled water (SDW) containing 0.05% Tween 80. The conidia concentration in SDW was determined for each isolate and adjusted to 10^7 spores ml^{-1}. SDW alone was used as the control. Filter papers with 12.5cm diameter were moistened with spore suspensions of *B bassiana* and placed in sterilized Petri dishes. To allow aeration, each dish was covered with a lid having a 2.5cm square hole in the center and sealed with a piece of plastic mesh. Five individuals of *S ingularis* comprising 4th - 5th instar nymphs and adults were released onto each treated filter paper. Each treatment was replicated five times. The insects were denied food for 24hrs in order to stimulate them to search for food by crawling all over the treated filter papers, thereby contaminating their bodies with the spores. They were transferred onto food (cocoa chupons) after the 24 hours and observed every other day for 15 days at 25 to 26°C. The following data were collected: (a) % mortality and (b) % of dead insects with external growth of *Beauvaria*. Mean % mortalities were corrected for natural mortality, using Abbotts' formula. Abbott.

Experiment 2

Only three isolates, IMI 335249, I97 1035 and I00 1183, were tested in the second experiment because of insufficient numbers of the mirid. The methodology and experimental conditions were largely the same as described above, except for the following modifications: The spore concentration of each test isolate was adjusted to 4 x 10 (Pham *et al.* 1995) ml^{-1} and *S singularis* was fed on fruits of *Desplastsia dewevrei* (Tiliales: Tiliaceae), reported to be more suitable for rearing the bug (Padi and Sarfo 2002). Ten insects were placed in each of four Petri dishes (replicates) lined with spore-contaminated filter paper, left undisturbed for four hours and thereafter transferred into plastic cages containing *D dewevrei* fruits. Mean % mortalities were recorded after 5, 10 and 15 days, as well as the number of dead insects with fungal growth and level of sporulation.

Experiment 3

In the 3rd bioassay, four isolates, IMI 335249, I97 1036, I97 1037 and I00 1183 were tested. Two milliliters of 4 x 10 (Pham *et al.* 1995) ml^{-1} concentration of each isolate were used to contaminate each of three filter papers (replicates). Three filter papers were treated with SDW plus 0.05% Tween 80 to serve as the control. Twenty *S singularis* were brushed into each bowl and left to crawl for one hour on the filter paper before being fed with a mixture of chupons and unripe cocoa pods.

Experiment 4

The final bioassay tested all the five isolates. Three batches (replicates) of four cocoa chupons each were contaminated (sprayed) with one of the five *B bassiana* formulations prepared as described above and placed in three different plastic bowls lined with untreated filter papers. The control treatment comprised chupons treated with SDW plus 0.05% Tween 80 only. Twenty *S singularis* were introduced onto the chupons in each plastic bowl.

RESULTS

During the two-season search for pathogens, *Fusarium* sp, *Aspergillus* sp, *Nomuraea* sp and *Ascheinrsonia* sp, as well as species of some unidentified fungi were isolated from capsids. *Cordyceps* sp and *Entomophaga* sp were isolated from ants (Hymenoptera: Formicidae) and *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae), respectively. It was not possible to recognize several other insects with fungal growth as they were completely covered by the fungal tissue. In any case, none of the fungi was bio-assayed since they were not amenable to exploitation in bio-control (Oduor, Pers comm)

In the 1st bioassay, isolate I97 1035 recorded the highest mirid mortality (71.4%) after 5 days, followed by I97 1036 (60%), IMI 335249 (48.6%), I00 1183 (31.4%) and I97 1037 (20%) (Table 1)

By the end of the 10th day, mortality levels had risen for all the isolates and isolate I97 1036 had already caused 100% mortality, while each of the other four had recorded 86.5% mortality. Three of the isolates caused 100% mortality after 15 days while two, including I00 1183 originating from Ghana, recorded 87.5% mortality.

Results of the 2nd bioassay (Table 2). Five days after the experiment was set-up, isolate I97 1035 had recorded the highest mortality (52%), followed by I00 1183 (44%) and IMI 335249 (40%). But by the 10th day IMI 335249 had caused the highest mortality (80%) followed by I97 1035 (68%) and I00 1183 (64%). Mortality levels remained unchanged thereafter. Isolate I00 1183 sporulated most profusely on the dead insects (Plate 1), followed by I97 1036 (Plate 2).

Results for 3rd and 4th experiments were equally promising. The % mortality level in the former was highest among *S singularis* treated with isolate I97 1037, followed by I00 1183, I97 1036 and then IMI 335249. Similar results were obtained in the 4th experiment, the highest % mortality being, once

Table 1. **Percent *D theobroma mortalities* 5, 10 and 15 days after exposure to isolates of *B bassiana*.**

B bassiana isolates	Corrected % mortalities		
	5 days	10 days	15 days
I97-1037	20.0	86.5	100.0
I97-1036	60.0	100.0	100.0
I97-1035	71.4	86.5	87.5
I00-1183	31.4	86.5	87.5
IMI 335249	48.6	86.5	100.0

Table 2. **Percent *D theobroma* mortalities and sporulation 5, 10 and 15 days after exposure to isolates of *B bassiana*.**

B bassiana isolates	Number of *D theobroma* treated	% Mortalities at day:			% producing spores
		5	10	15	
IMI33 5249	10	40.0	80.0	80.0	20.0
I97-1035	10	52.0	68.0	68.0	68.0
I00-1183	10	44.0	64.0	64.0	12.0

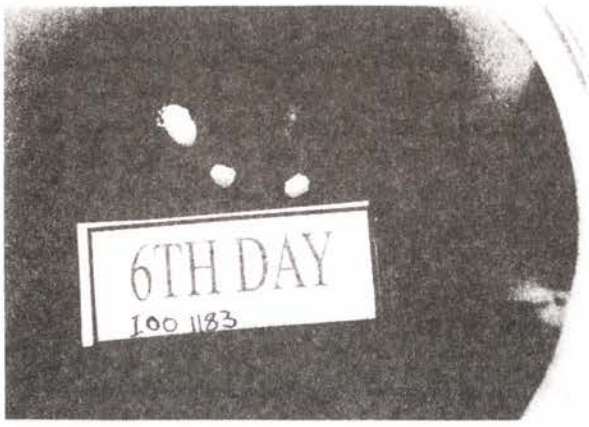

Plate 1. **Extent of sporulation by *B. bassiana* isolate I00 1183 on *S. singularis* six days after treatment.**

Plate 2. **Extent of sporulation by *B. bassiana* isolate I97 1036 on *S. singularis* six days after treatment.**

again, recorded on *S singularis* contaminated with isolate I97 1037 followed by I00 1183. However, *S singularis* treated with IMI 335249 had greater mortality than those treated with isolates I97 1035 and I97 1036.

DISCUSSION

The control of cocoa mirids in Ghana is mainly by the use of chemical insecticides such as Confidor, Cocostar and Carbamult. Although these are effective, the risk of pest resistance, residual toxicity, environmental concerns and the high cost of treatments make it imperative to look for alternative products such as natural enemies, including pathogens. The present study has demonstrated that *B bassiana* is a potential biological agent for the control of *S singularis*, and probably other cocoa mirids. The 20 to 71.4% mortality rates in the first experiment, and the 40 to 52% in the second, 5days after treatment, together with even higher mortalities after 10 and 15 days in the experiments clearly attest to the effectiveness of the isolates, particularly isolate I97 1035.

The mortality rates in experiment 1 were significantly higher than that in experiment 2. This disparity is difficult to explain, but it may be due to differences in the viability and, therefore, the persistence of *B bassiana* conidia in the two formulations (Godonou *et al.* 2000). Another possible cause for the disparity was the different food sources for the two experiments; i e, cocoa chupons for the 1st experiment and fruits of *D dewevrei* for the second. In any case, the results point clearly to the great potentials of *B bassiana* as bio-control agent for *S singularis* on cocoa.

The exact mode of mirid infection by *Beauvaria* is unknown and studies in this area will be useful. However, it is apparent in this study that infection of *S singularis* occurred through the mouthparts, the abdominal segments and the antennae as indicated by fungal growth and sporulation patterns.

Profuse sporulation of the isolates on the dead mirids was clearly evident in the 2nd bioassay. Thus, all the dead insects were completely covered with the fungal spores 10days after spraying. This suggests the possibility of healthy (uninfected) individuals being infected in a field situation through such profuse sporulation on infected (dead) individuals. *B bassiana* is known to be non-fastidious, growing and sporulating on a wide variety of media. Lim *et al.* (1989). In this work, all the test isolates grew and sporulated well at room temperature, i e 25 - 26 ° C. Although further investigations are required in these areas, it is apparent from the data that the cocoa ecosystem in Ghana will support the growth and sporulation of *B bassiana* and thereby enhance its persistence in the target environment. This will ultimately keep the *S singularis* population down without necessarily re-applying the control agent (Godonou *et al.* 2000).

CONCLUSIONS

The present study has demonstrated clearly the potentials for *B bassiana* isolates in the management of *S singularis* numbers on cocoa. Ultimately, microbial control could form an important

component of an IPM strategy against the mirid on cocoa. Implementation will, however, require in depth research in areas of mass-production, formulation and delivery. Further tests on human safety, virulence to target pests, safety of non-target fauna and persistence, among others, will also have to be thoroughly investigated.

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ACKNOWLEDGEMENTS

The authors are grateful to the field staff of both the Entomology and the Plant pathology divisions of CRIG for the collection of insect specimens, and also to Ms Mercy Ofori and Mr A Nkansah for the laboratory work. The work was sponsored by DFID, NRI and CABI-Bioscience. This paper is published with the kind permission of the Executive Director of the Cocoa Research Institute of Ghana.