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THE POTENTIAL OF *Beauveria bassiana* FOR THE MANAGEMENT OF *Cosmopolites sordidus* (Germar, 1824) ON PLANTAIN (*Musa*, AAB)

A THESIS SUBMITTED TO THE
DEPARTMENT OF CROP SCIENCE,
UNIVERSITY OF GHANA, LEGON

BY
IGNACE GODONOU

IN PARTIAL FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

OCTOBER 1999

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DECLARATION

I do hereby declare that, except for references to works of other researchers which have been duly cited, this work is the result of my own original research and that this thesis either in whole or in part has not been presented for another degree elsewhere.

I. GODONOU
(STUDENT)

DR K. A. ODURO
(SUPERVISOR)

PROF. K. AFREH-NUAMAH
(SUPERVISOR)

DR K. R. GREEN
(SUPERVISOR)

INTEGRI PROCEDAMUS

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ACKNOWLEDGEMENTS

It is a real pleasure to express my heartfelt gratitude to my supervisors Dr K. A. Oduro and Professor K. Afreh-Nuamah both at the University of Ghana, Legon and Dr K. R. Green at the International Institute of Tropical Agriculture (IITA) who guided me throughout the course of this study. I am extremely thankful to them for all the time and care they devoted to this work.

I would like to thank Professor E.V. Doku, for guiding me through my work and helping me to solve other problems at the University.

I am very grateful to Professor J.N. Ayertey Head of Crop Science Department, University of Ghana, Legon, for accepting me as student and for academic supervision. My thanks are extended to all the lecturers at Crop Science Department who took a keen interest in my work and who were always helpful to me. My thanks also go to the administrative staff of the University of Ghana for help that made me go through regulations at the University.

I am greatly indebted to Drs C.J. Lomer, J. Langewald, C.S. Gold and P. Speijer of IITA for their dedication to this research and for their useful suggestions and innovative help with the Methodology.

I wish to thank Dr P. Neuenschwander, Director of Plant Health Management Division (PHMD)/IITA and Dr. C.J Lomer, scientist/Leader at IITA/PHMD for releasing me from IITA and recommending me to undertake my course work at the University.

My sincere thanks to Dr I. Ofori of Crop Science Department of the University of Ghana and Mr S. Korie of the International Institute of Tropical Agriculture (PHMD) for their useful advice and guidance with data analysis.

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Thanks to Drs J. K. Osei and K. G. Ofosu-Budu and Mr E. K. S. Ahiekpor at the University of Ghana, ARS-Kade for their comments and suggestions throughout the course of this study.

Thanks to Eric Cornelius of the Crop Science Department for always being with me and guiding me from my course and research work to the end of the thesis writing.

Special thanks to my Technician, Mr. Aseidu Bismark for the nice job he did with me during my research work.

To the staff of the West African Plantain Project especially Messrs. Nsiah, Banfo, Brentu, Alex Eppi, Musa, Henry and Lambert, I say a big thank you for your support.

Many thanks also go to all the LUBILOSA local staff in Cotonou; Pierre Affa, O. K. Douro-Kpindu, Gbongboui Comlan, Serge Attignon, Gabriel Heviefo, Olga Idohou, Léonard Nassara, Theotime Adouhoun, Romain Houénoussi, Etienne Dagbozounkou, Noèlie Dossou, Yves Amoussougbo and Coopéri Gnanhoui for providing me technical assistance in IITA, Benin

I wish to express my sincere thanks to Mr Sampson Nyampong for the useful advice and moral support he gave me during the course of this work.

Thanks to Dr Georgen and Mr. David both of IITA/PHMD for identification of some field collected insects

I wish to express my thanks to Mr Ashian and his wife and Mr Ntri for their social assistance to me during my stay in Kade, Ghana.

The project was funded by the German Federal Ministry for Economic Cooperation and Development (BMZ) and administered by the West African Plantain Project, IITA. The support of these organisations is gratefully acknowledged.

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LIST OF ABBREVIATIONS

Anon.	Anonymous
ANOVA	Analysis of variance
ARS	Agricultural Research Station
ARSEF	Agricultural Research Service Collection of Entomopathogenic Fungi
BHC	Benzene Hexachloride
CABI	CAB International
CP	Conidial powder
CS-CP	Clay soil-based formulation of conidial powder
DDT	Dichloro Diphenyl Trichloroethane
df	Degrees of freedom
e.g.	For example
F	Fisher's test
GDP	Gross Domestic Product
GLM	General linear models
GO-CP	Groundnut oil-based formulation of conidial powder
GOK-CP	Groundnut oil plus kerosene-based formulation of conidial powder
i.e.	That is
IITA	International Institute of Tropical Agriculture
IMI	International Mycological Institute
IPM	Integrated Pest Management
LUBILOSA	Lutte Biologique contre les Locustes et Sauteriaux
MS	Mean of Square
OPKC	Oil palm kernel cake
OPKC-C	Oil palm kernel cake-based formulation of conidia
P	Probability
PDA	Potato Dextrose Agar
PHMD	Plant Health Management Division
SAS	Statistical analysis system
SDA	Sabouraud Dextrose Agar

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ABSTRACT

The banana weevil, *Cosmopolites sordidus* (Germar) in association with other pests and diseases, represents a threat to the production of plantain (*Musa* spp., AAB), the preferred staple food in Ghana. Biological control of the banana weevil was considered the most promising management option for small-scale plantain production and studies were, therefore, undertaken to determine the efficacy of the entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin in the management of *C. sordidus*.

The duration and spatial distribution of the different developmental stages of *C. sordidus* within plantain plants were determined to provide background information for evaluation of *B. bassiana* against the banana weevil. The mean egg incubation period and the mean developmental period from larva to pupa and pupa to adult were 6.3 ± 0.2, 28 ± 0.6 and 7.1 ± 0.3 days respectively. The developmental period from egg to adult ranged from 33 to 51 days with a mean of 40.4 ± 0.7 days. Within the plantain plant, approximately 80% of the eggs were located in the rhizome, >80% of the larvae were found at the rhizome level and all of the pupated larvae were located in the rhizome, suggesting that this is where a biocontrol agent should be targeted, rather than the pseudostem.

Three strains of *B. bassiana* were obtained and evaluated on the basis of virulence tests and potential for mass production. From the results of these tests, strain IMI330194 of *B. bassiana* was selected for subsequent studies. Laboratory studies using a water-based inoculum applied to corm pieces or pseudostem traps, showed that *B. bassiana* could control all stages of *C. sordidus*, with up to 21.3%, 36.4% and 42.3% of eggs, larvae and adults respectively showing signs of fungal disease. Pot experiments to compare different formulations of the strain IMI330194 against adult weevils showed that the highest mortality (>60%) was obtained with groundnut oil plus kerosene-based formulation of conidial powder (GOK-CP), groundnut oil-based formulation of conidial powder (GO-CP) and oil palm kernel cake-based formulation

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of conidia (OPKC-C). A persistence trial showed that OPKC-C of IMI330194 still gave 61.0% weevil mortality one month after application, compared with only 12.3% for conidial powder (CP) of IMI330194 and 3.9% for the control with no conidia. In field trials with artificial weevil release, mortality of adult weevils exposed to CP and OPKC-C of IMI330194 ranged from 53.4 to 75.5%, compared to <8% in the control with no conidia. Under natural weevil infestation, 16.7% of plantain suckers treated with CP of IMI330194 and 19.4% of untreated suckers were killed by weevil attack. In contrast, none of the suckers planted with OPKC-C of IMI330194 were killed. A study on the spread of fungal conidia using artificially infected and non-infected adult weevils showed a possible dissemination of *B. bassiana* conidia from infected weevils up to 18 m from the release point. On the basis of results from the present study, the strain IMI330194 of *B. bassiana* could clearly play a key role in the management of *C. sordidus* adults on plantain.

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CHAPTER 1

GENERAL INTRODUCTION

Plantain (*Musa* spp. AAB) is a particularly important crop for farmers in the humid forest agro-ecological zone of West and Central Africa (Anon., 1990) where approximately 43% of the world's plantain is produced (Anon., 1993). Because of its long history of wide spread cultivation and distribution, the region has become a secondary centre of plantain diversity (Swennen and Vuylsteke, 1990). It is estimated that about 70 million people in West and Central Africa derive greater than one-quarter of their food energy requirements from plantain, making it one of the most important sources of carbohydrate throughout the African lowland humid forest zone (Swennen, 1990). Unlike sweet dessert bananas, plantain is a staple food which is fried, boiled and sometimes pounded or roasted and consumed alone or together with other food (Swennen, 1990; Afreh-Nuamah and Hemeng, 1993).

In addition to being a staple food for rural and urban consumers, plantain provides an important source of income for resource-poor farmers (Anon., 1993). Plantain is a highly priced staple crop in Ghana. The crop is mainly grown by small-scale subsistence farmers who produce an average of 7.1 tonnes per hectare (Anon., 1991a). In terms of national crop production, plantain is ranked fourth among the major crops in Ghana and accounts for 9% of agricultural Gross Domestic Product (GDP) (Anon., 1991a). As plantain is a major staple in Ghana, a shortfall in production results in scarcity on the general market and a corresponding price increase (Schill *et al.*, 1997)

In the traditional cropping systems of Ghana, plantain is grown together with root crops, cereals and vegetables or as shade for tree crops, like cocoa (Afreh-Nuamah, 1993a). As a backyard crop, it also does well, coexisting easily with established farming systems. It can provide a continuous source of food over the cropping year and counteracts degradation of the soil through the prolific leaf mulch cover it produces.

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The yield of banana and plantain in tropical and subtropical regions, where these crops are a major source of food and revenue, is adversely affected by diseases and pest infestations (Simmonds, 1966). Out of about two hundred insect pests recorded attacking *Musa* spp., the most important is the banana weevil, *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae) (Purseglove, 1972). The damage done by the weevil is primarily the result of destruction of corm tissue, sometimes accentuated by secondary attacks by other insects and micro-organisms (Simmonds, 1966) leading to increased risk of toppling. In Ghana, the combined effect of nematode and banana weevil infestations on plantain can lead to yield losses of 85% in the plant crop (Udzu, 1997). In Uganda, banana weevil outbreaks can cause up to 100% yield losses (Sengooba, 1986; Sebasigari and Stover, 1988).

Limited control of the banana weevil is achieved by use of cultural, biological and chemical strategies. In the absence of any better control strategy, many national programmes world-wide adopt and recommend chemical pesticides as the main control strategy to farmers (K. Afreh-Nuamah, personal communication, 1998). In the short term, chemical pesticides are expensive and provide only temporary relief. Moreover, reproductive and evolutionary capacities of the insects allow the pest to develop mechanisms of resistance to the chemicals (Metcalf, 1980). The detrimental effects of pesticide application on human health, damage to non-target organisms and possible environmental pollution of residential and agricultural lands, and of ground water suggest that the use of chemicals has to be evaluated alongside other methods. For these and other reasons, biological control is now receiving considerable attention.

Several natural enemies of *C. sordidus*, mainly in the orders Coleoptera and Diptera, have been identified and attempts to manage the population of *C. sordidus* using introduced predators and parasitoids have been made. The Histerid beetle (*Plaesius javanus* Erichson (Coleoptera: Curculionidae)), which is native to Java, has already been successfully introduced in many countries as a biological control agent of *C. sordidus,* but in Africa this predator and many other predaceous beetles tested have not been effective (Nankinga, 1994). In Cuba, *Tetramorium guineense* Auct (= *T.*

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bicarinatum Nylander) (Hymenoptera: Formicidae) has been used successfully to reduce the weevil population by 65% in heavily infested plantations and by 83.5% in less heavily infested ones (Roche and Abreu, 1983). Treverrow *et al.* (1991) reported that in Australia the entomopathogenic nematode *Steinernema carpocapsae* gave significant mortality of *C. sordidus* larvae in rhizomes (16-68% depending on the larval size and mode of nematode application). In Brazil, the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin have also shown good control against adults of *C. sordidus* under laboratory conditions (Batista-Filho *et al.*, 1987; Busoli *et al.*, 1989). Laboratory infectivity tests conducted using different strains of *B. bassiana* isolated from dead banana weevil and soil samples in East Africa (Nankinga, 1994) and in West Africa (Traoré, 1995) gave good results of up to 100% weevil mortality. *B. bassiana* therefore, represents a promising alternative for the management of *C. sordidus* in Africa.

For sustainable management of *C. sordidus* with an entomopathogenic fungus there is a need to know more about aspects of its biology such as the developmental period of its different stages, the spatial distribution of the different stages within a standing plant and the movement of the adult in the field. Factors affecting the success of any microbial agent for the control of an insect pest include the selection of an appropriate agent (i.e. most virulent strain), the potential of the strain for mass-production, development of an efficient and cost effective formulation and delivery system, and the feasibility of the control measure in the small-scale farmer's context.

The present study therefore, aims to:

(i) Determine the duration of the developmental period of different stages of *C. sordidus* in Ghana,

(ii) Determine the spatial distribution of the different stages of *C. sordidus* on plantain,

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(iii) Select virulent strain (s) of *B. bassiana* with good production characteristics for the management of *C. sordidus*,

(iv) Develop and evaluate different formulations of *B. bassiana* against adults of *C. sordidus* in pot experiments,

(v) Evaluate the selected formulation of *B. bassiana* against adults of *C. sordidus* in the field.

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CHAPTER 2

LITERATURE REVIEW

2.1. Origin and distribution of plantain and banana

Plantain and banana are from the family Musaceae in the order Zingiberales (Purseglove, 1972). The family Musaceae has two genera, *Musa* and *Ensete*. *Ensete* is an old and declining genus, which probably originated in Asia and spread to Africa and is now equally divided between both continents (Purseglove, 1972). *Musa* is a rhizomatous herb which originated in South-eastern Asia and the Pacific, with its centre of diversity and probably of origin in the Assam-Burma-Thailand area (Purseglove, 1972). *Musa* is the largest, most widely distributed and most diversified genus of the family, consisting of up to 15 known species (Simmonds, 1982). Edible *Musa* cultivars are from two wild species, namely *Musa acuminata* Colla (A genome) and *Musa balbisiana* Colla (B genome) (Simmonds, 1982). *Musa* spp. are grown mainly in the lowland tropics (Purseglove, 1972). Simmonds (1982) believed that the dispersal of AAA clones and hybrid cultivars (AAB and ABB) was from northern India by the way of Saudi Arabia and the Horn of Africa.

The cultivation of plantain (*Musa* spp. type AAB), and highland banana (type AAA, AB, ABB) is limited to within 30^0 north and south of the equator (Purseglove, 1972). Plantains are grown widely in Côte d'Ivoire, Ghana, Nigeria, Cameroon, Congo and Zaire, while cooking bananas (*Musa* spp. type AAA-EA) and beer bananas (*Musa* spp. types AAA-EA, AB, ABB) are produced in Uganda, Rwanda, Burundi and northern Tanzania (Gold, 1993).

2.2. Botany of plantain and banana

Musa spp. are tree-like giant perennial herbs with an underground stem or corm, a pseudostem composed of leaf sheaths, and a terminal crown of leaves through which the inflorescence emerges (Purseglove, 1972). The underground stem has extremely short internodes covered externally by closely packed leaf scars, which completely

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encircle the corm. The aerial part or pseudostem is composed of leaf sheaths borne in the left-handed spiral with a phyllotaxy of one-third on young suckers and a terminal crown (Purseglove, 1972). The pseudostem is functionally the trunk of the plant, varying in height from 2 m to 8 m depending upon cultivar, and which later supports the fruit bunch.

2.3. Production and economic importance of plantain in Ghana

Ghana is the fifth largest plantain and banana producer in West and Central Africa, Nigeria being the first followed by Zaire, Cameroon and Côte d'Ivoire in that order (Anon., 1993). Of a total of 7,960,000 tonnes produced by West and Central African countries, Ghana produced 8% with a per capita consumption of plantain of 60 kg a year (Anon., 1993). The total area cropped to plantain is about 129,000 ha (Anon., 1991a).

Plantain is extensively grown throughout the forest zone of the country and can be found in backyard gardens of most houses. Akomeah *et al.* (1995) gave the distribution of plantain production in different regions of Ghana as follows:

Table 1. Plantain production in different regions of Ghana.

Region	Land area (hectares)	Yield (tonnes/hectare)
Ashanti	50,940	6.8
Eastern	39,200	8.3
Western	31,000	6.2
Brong-Ahafo	24,700	5.5
Central	6,600	5.5
Volta	4,600	6.5

Source: Akomeah *et al.* (1995)

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In Ghana, plantain is the fourth most important starch-staple after grains, cassava, and yam (Cropley and Morriss, 1993). It is a highly priced crop, reflecting a strong consumer preference and huge demand (Schill *et al.*, 1997). The bulk of the crop is grown in the southern part of the country with rainfall of about 1500 mm per annum and an annual water deficit below 400 mm (Ahiekpor, 1996). In the producing areas, any meal prepared without plantain, cocoyam, or yam is not considered as a full meal. In spite of its cultural value, plantain production has declined during the past two decades (Akomeah *et al.*, 1995).

2.4. Constraints to plantain production

A wide range of factors limits plantain and banana productivity. These factors include poor crop and soil management practices, inherent low soil fertility, reduced fallow periods (due to increasing population and food demand), inadequate supply of good quality planting materials, low yield potential for most local varieties, high post-harvest losses, and an extensive pest and disease complex (Karikari, 1970). The relative importance of constraints varies within Africa. In Uganda for example, banana weevil is the key constraint followed by nematodes and black Sigatoka (a foliar disease caused by the fungal pathogen *Mycosphaerella fijiensis* Morelet) (Gold *et al.*, 1993), while in Southern Cameroon, the major constraints in order of importance are, black Sigatoka, nematodes (*Radopholus similis* (Cobb) Thorne) and *C. sordidus* (Anon., 1997). Farmers in Ghana have identified several constraints to plantain production (Schill *et al.*, 1997) among which nematodes, banana weevil, weeds and black Sigatoka are the most important.

The major factor determining the productivity of banana and plantain is the health of the root system, which is responsible for nutrient and water uptake (Swennen, 1986). According to Bridge *et al.* (1993), nematode species known to cause the most serious root damage to *Musa* spp. are *R. similis*, *Pratylenchus coffeae* (Zimmermann) Filipjev and Steckh., *Pratylenchus goodeyi* Sher and Allen, and *Helicotylenchus multicinctus* (Cobb) Golden.

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In Ghana, Schill *et al.* (1996) reported that *P. coffeae*, *H. multicinctus* and *Meloidogyne* spp. are the most wide-spread nematodes species on plantain. The first two species occur at high densities while the latter occur at low density. The dominant nematode species in Ghana are all lesion-forming nematodes.

Damage to the roots infected by plant parasitic nematodes and the resulting severe root-rot caused by secondary invasion of fungal pathogens are considered to be responsible for a major part of toppling of banana and plantain and subsequent yield loss (Stover, 1966; Sikora and Schloesser, 1973). The feeding activity of some plant parasitic nematodes on the root system causes root necrosis. Plants with necrotic roots are less able to take up water and nutrients resulting in stunted growth, delayed maturation time and reduced bunch size (Speijer *et al.*, 1994). Udzu (1997) reported a yield reduction of 63.2% due to nematodes. A combined infestation of *C. sordidus* and nematodes reduced yield by more than 80% (Anon., 1997; Udzu, 1997).

The larvae of *C. sordidus* bore into the corm and the pseudostem, causing mortality of suckers, snapping (Feakin, 1971; Koppenhöfer, 1993) and toppling (Bosch *et al.*, 1995; Pena *et al.*, 1993; Rukazambuga, 1996) of the pseudostem. Extensive tunnelling by the larvae interferes with root initiation as a result of the destruction of the corm's cortical tissue. This leads to the production of a small number of roots, which consequently affects the anchorage of the plant (Wright, 1977) resulting in a general decrease in productivity (Pena *et al.*, 1993).

Damage is usually greater in ratoon crops (Mitchell, 1978; Taylor, 1991; Gold *et al.*, 1994; Pone, 1994). Heavily infested plants produce small bunches, and have reduced resistance to drought and strong winds may sweep many large or maturing plants down (Sikora *et al.*, 1989). Such "blow-downs" or "toppling" can lead to crop losses ranging from 50 to 100% (Hord and Flippin, 1956; Stover, 1966). In heavily infested plantations, the suckers produced are weak (Froggatt, 1925).

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2.5. Origin and distribution of *C. sordidus*

C. sordidus is believed to have originated in Southeast Asia, probably the Malaya/Java/Borneo region, as it is there that the weevil's natural predators were found (Kranz *et al.*, 1977). The banana/plantain weevil has gradually spread with banana/plantain stalks to various parts of the world (Harris, 1947). In 1900, the insect was recognised in the Far East, Australia and Brazil and during the following 20 years, was observed in Central Africa, Central America, the Pacific Islands, the Indian Ocean Islands and the Caribbean (Kranz *et al.*, 1977). The banana weevil now has a pan-tropical distribution and is recognised as an important pest of *Musa* spp. in most production areas of plantain and banana (Hill, 1983; Neuenschwander, 1988). The banana/plantain weevil reached pest status between 1960s-1970s, more than 60 years after being introduced to Africa and is now widely disseminated (Anon., 1991b). The factors underlying changes in *C. sordidus* pest status remain undetermined.

2.6. Morphology and biology of *C. sordidus*

The adult weevil is soft and brown when newly emerged from the pupal case and later changes from dark-brown to black in colour. The adult weevil has a long slightly curved trunk in front of the head and is approximately 12.5 mm long and 4 mm in width (Lescot, 1988). The mature adult lays its eggs in the standing stem of the growing plant or the stump of a recently harvested plant (Whalley, 1957). The female bites a small hole in the corm at the ground level and after preparing an incubation chamber, deposits a single egg (Kranz *et al.*, 1977). Oviposition takes place at night and occurs throughout the year (Woodruff, 1969) but is greatest during the rainy season. A single female can lay up to 100 eggs during its lifetime but usually the number does not exceed 50 (Kranz *et al.*, 1977).

The eggs of *C. sordidus* are about 2 mm long, oval in shape and white in colour (Whalley, 1957, 1958). The duration of the various weevil stages varies widely according to season and locality (Cuillé, 1950). For example, the duration of the egg incubation period is 5-7 days in America (Moznette, 1920), 6-7 days in Jamaica

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(Edwards, 1934), 5-8 days in Uganda (Bakyalire and Ogenga-Latigo, 1992), 3-7 days in Ghana (Afreh-Nuamah, 1993b) and 4-33 in Queensland (Froggatt, 1924). After emergence, the larvae tunnel into the corm for 20 to 100 days but after a series of moults turn toward the periphery to pupate (Kranz *et al.*, 1977). The larval stage is reported to last between 14 and 21 days (Hill, 1983), 30-40 days (Seshu Reddy, 1986), 55-65 days (Bakyalire and Ogenga-Latigo, 1992) or 11-16 days (Afreh-Nuamah, 1993b). The duration of the developmental period from larva to pupa decreases as temperature increases (Traoré, 1995). The pupal stage is reported to last 5 to 8 days (Harris, 1947; Whalley, 1957; Woodruff, 1969), or 8 to 10 days (Bakyalire and Ogenga-Latigo, 1992; Afreh-Nuamah, 1993b). The mature adult often remains longer within the plant before biting the external sheath (Cuillé, 1950). The life cycle of the weevil has commonly been reported to last 30 to 50 days (Wolfenbarger, 1964; Woodruff, 1969; Hill, 1983) but studies by Bakyalire and Ogenga-Latigo (1992) in Uganda have shown a longer duration ranging between 53 and 72 days.

2.7. Life history of *C. sordidus*

The adult weevils live in the soil, feeding on rotten materials of banana and plantain and visiting the growing plant for oviposition. They are sluggish and nocturnal in habit (Cuillé, 1950) and are negatively phototactic being more active in hours of darkness and positively hydrotactic, preferring high humidity areas (Ittyeipe, 1986). The weevil is known to live up to two years or more (Whalley, 1957; Wolfenbarger, 1964; Hill, 1983; Waterhouse and Norris, 1987). It is also known to survive for long periods without food (Woodruff, 1969), and a maximum of 180 days has been reported (Mitchell, 1980).

The spread of the weevil within banana-growing areas generally happens in three ways; in planting material from infested plantations (most important), by crawling from adjacent infested plantations, or by rain. Since banana and plantain may be planted on steep slopes, flood water carries infested materials as well as adult weevils from high to low areas (Kranz *et al.*, 1977). The adult weevils have functional wings but have rarely been observed to fly (Cuillé, 1950). The banana weevil was observed

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to spread its functional wings without flight in the laboratory in dry conditions or when exposed to insecticides (Roth and Willis, 1963). Some authors have maintained that weevils never fly but Ostmark (1975) reported free-flight in Fiji on warm humid nights in the dry season, and it may also be a significant means of spread.

2.8. Host range and damage caused by *C. sordidus*

C. sordidus attacks only members of the genus *Musa* (Froggatt, 1925). The banana weevil was recorded on sugarcane, *Saccharum officinarum* (L.) Lamk and yam (*Dioscorea* spp.) but appears to be only a very minor pest on those crops, perhaps attacking them only when plantain and banana are not available (Woodruff, 1969). *C. sordidus* has also been reported on sweet potato tubers, *Ipomoea batatas* (L.) Lamk (Cuillé, 1950).

During dry weather conditions, when the plants are striving against adverse growing conditions, the effect of the borer undermining the vitality of the plants brings about a more or less complete breakdown of the stools far more rapidly than would occur in a normal season (Froggatt, 1925). The larva is a voracious feeder, devouring an amount of tissue equal to many times (5 to 10) its own body weight per day (Cuillé, 1950).

Plant losses due to the attack of banana weevil depend on the stage of the plant, and the level of the weevil infestation in a field. According to Karamura and Gold (1996), the banana weevil damage has three main effects. Firstly, the infected plants are denied the ability to produce roots and normal suckers because of the destruction of the cortical tissue of the corm. Thus the affected plants are unable to absorb sufficient nutrient and water and produce "water" suckers which rarely reach the flowering stage. Secondly, larval tunnels may destroy the central cylinder (with vascular bundles). When this happens, the physiological communication between the aerial shoot and the underground stem is cut and, depending on the growth stage, the plant may die or produce poor quality roots and reduced yield. Thirdly, weevil tunnels may affect the meristem region in which case growth will halt, leading to failure of leaf production and death of the plant. It was reported that in a situation of heavy

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infestation, mature plants may be killed or fail to flower while newly planted suckers within infested fields are readily destroyed almost immediately they are planted (Karamura and Gold, 1996). In Uganda for example 60% of the suckers planted in heavily infested fields may die due to weevil attack (P. Speijer, personal communication, 1997). In West and Central Africa a range of damage levels and yield losses have been reported. For example, in Côte d'Ivoire, yield loss was correlated with intensity of attack and reductions of 30 to 60% were found to be common (Vilardebo, 1973). Lescot (1988) reported yield reductions of 20 to 90% in Cameroon. In Ghana, Afreh-Nuamah (1993a), reported that one month after planting, percentage weevil infestation ranged from 0 to 82.5% depending on the origin of planting material (i.e. nursery material or ratoon material), history of land (cropped land or forest land) and cultivars. Also, Udzu (1997) reported a yield reduction of 33.3% due to weevil infestation only and 86.1% yield reduction when the effect of nematodes and weevil infestation was combined.

2.9. Control of *C. sordidus*

Several methods are used to control the banana weevil. These include cultural, chemical and biological methods (Whalley, 1957; Simmonds, 1966; Hill, 1983).

2.9.1. Cultural control

Cultural control involves destroying the sheltering and feeding places of the adult weevils. Pseudostems from which bunches have been harvested are cut at the ground level, chopped up and scattered in the plantation, so that they dry off or rot as quickly as possible. The cut face of the old corm could also be covered with soil (Simmonds, 1966).

Weed control is also important, especially in the immediate vicinity of the banana stools. Since the adult weevils are sluggish and nocturnal in habit (Cuillé, 1950), bare soil or soil with a low density of weeds reduces their movement, thus resulting in a reduction in stool infestation.

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At planting, care should be taken not to use infested suckers. Suckers should be taken from a field known to be free of the banana weevil. If this is not possible, the sucker should be closely inspected and, if necessary pared (by removal of the outer tissue layer of the rhizome) to remove the unhatched eggs and larvae (Harris, 1947; Whalley, 1957; Saouder, 1961; Feakin, 1971). When suckers have been dug they should be removed from the field at once and not left in heaps overnight. It has repeatedly been suggested that eggs and larvae in infested suckers can be killed by soaking the suckers in water for one to two days, however, it has been conclusively shown that only a very prolonged period of soaking leads to disinfested planting material. In practice, therefore water soaking of sucker is useless (Simmonds, 1966). Hot water treatment leads to less than 35 % larval mortality at 54°C for 20 min or 60°C for 15 min and more than 90% mortality at 43°C for 3 hours (C.S. Gold, personal communication., 1996).

Good crop husbandry, involving such practices as clean weeding, desuckering, pruning, manuring and mulching produces vigorous plants that are more tolerant to weevil damage (Feakin, 1971). Good crop management (inter-cropping, soil amendment, sanitation) was found to reduce weevil infestation (Gold *et al.*, 1997). For example, the same authors reported that banana fields inter-cropped with coffee coupled with good sanitation (i.e. weeding) and farms where crop residues were systematically destroyed, had a lower weevil population and lower weevil damage respectively than those having little or no crop sanitation.

Trapping is also an important cultural method for controlling *C. sordidus*. The use of pseudostem and rhizome traps is common practice in the removal of adult weevils from infested plantations. Pseudostem traps can either be a pseudostem split lengthways or a disc (Mitchell, 1978; Bujulu *et al.*, 1983). Traps are placed at the base of plantain or banana plant stools and the weevils that are attracted to them are collected and destroyed (Hord and Flippin, 1956). The frequency of collection varies widely, ranging from daily to weekly but all authors seem to agree that collection should be carried out not less than once a week (Simmonds, 1966). It was shown that

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the younger the trap, the better the attraction, which falls markedly after one week (Hord and Flippin, 1956). It was found that rhizome traps were more attractive to the banana weevil than pseudostem traps (Hord and Flippin, 1956). Trapping is often of limited effectiveness against established infestation and is tedious and time consuming. Nevertheless trapping may be cheap and effective when used in combination with chemical or biological insecticides in an integrated weevil management programme (Whalley, 1957; Feakin, 1971; Kranz *et al.*, 1977; Schmitt *et al.*, 1992).

2.9.2. Chemical control

Chemicals (synthetic insecticides) can be used for the control of banana weevil and are mostly used by commercial banana and plantain producers. They can be incorporated into traps or applied to soil (Simmonds, 1966). Insecticides, such as Paris green (alone or mixed with flour as bait), Benzene Hexachloride (BHC), Dichloro Diphenyl Trichloroethane (DDT) and others have been found to be repellent to the banana weevil and in any case, when applied in a field, do not reach the whole population (Simmonds, 1966). To improve the poison trapping technique, Cuillé (1950) devised a mixture containing two insecticides (chlordane and parathion) with an attractive chemical carried in an inert dust. Ogenga-Latigo and Mazanza (1996) reported population reduction of *C. sordidus* by incorporating chemical insecticides to pseudostem traps. In general, although poison traps kill some borers, they appear to be relatively ineffective and severe infestation cannot be controlled by this means alone (Simmonds, 1966). Soil treatment with insecticides is based on the principle of killing the egg-laying females on their way to plantain or banana plants. To achieve control by this means, the insecticide needs to be sufficiently persistent in the soil to avoid the need for frequent re-treatment (Simmonds, 1966). Persistent organochlorine, such as DDT and Dieldrin were until the 1970's the standard recognised chemicals used against *C. sordidus* (Nankinga, 1994). Since then, however, environmental concerns such as pollution of agricultural lands and ground water and the development of resistance by the weevils to the organochlorine chemicals in many countries (Mitchell, 1980), necessitated a change to organophosphate and/or carbamate insecticides, such

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as Primiphosethyl (Primicid) and carbofuran (Furadan) which are less persistent and less harmful to non-target organisms (Edge *et al.*, 1974, Nankinga, 1994).

The high cost and scarcity of synthetic insecticides have limited the farmers ability to acquire and use them. Farmers who can afford to buy chemicals tend to apply inadequate doses (under-dosing), a situation that renders them ineffective and enhances probability of pests developing resistance to chemicals (Nankinga, 1994). Chemical control methods are, therefore, environmentally and socio-economically unsound in the context of the resource-poor smallholders who mainly grow the crop in Africa.

2.9.3. Classical biological control of *C. sordidus*

Attempts to control *C. sordidus* using introduced predators and parasitoids have been documented for a long time in different localities. The natural enemies of *C. sordidus* include *P. javanus*, *Hyposolenus laeviganus* Marseul (Coleoptera: Histeridae), *Hololepta quadridentata* Fabricius (Coleoptera: Histeridae), *Belonochus ferrugatus* Erichson (Coleoptera: Staphylinidae), *Priochirus unicolor* L. (Coleoptera: Staphylinidae), *Cathalus* spp. (Coleoptera: Silvanidae) and *Dactylosternum hydrophiloides* Macleay (Coleoptera: Hydrophilidae). The known diptera controlling the banana weevil is *Chrysopilus ferruginosus* Wiedemann (Diptera: Rhagionidae) (Neuenschwander, 1988). Some ants were recorded preying on the larvae of the banana weevil. These were *Anochaetus* sp. (Hymenoptera: Formicidae) (Seshu-Reddy, 1986) and *T. guineense* (Roche and Abreu, 1983).

Previous efforts to control the banana weevil through the release of natural enemies were inadequate and met with only limited success. For example *P. javanus* was imported from Java into Uganda in 1934, and released on Kibibi Island in Lake Victoria in 1935. The release site was visited in 1944 but no *P. javanus* was found, and it was presumed not to have established (Harris, 1947). *P. javanus* and *H. quadridentata* were released in Cameroon and were not able to establish (Greathead *et al.*, 1971). *P. javanus* was introduced in the Pacific Region, Trinidad, Australia and

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most of South America and was found to establish (Greathead *et al.*, 1971). However, the natural enemies used were generalists, not specific to the banana weevil and releases were small and disregarded the possibility of biotypes (Waterhouse and Norris, 1987; Neuenschwander, 1988; Greathead *et al.*, 1971). In Africa, one of the possible reasons for the failure of the exotic natural enemies to establish may be their non-adaptability to the environment. The greatest potential for the biological control of *C. sordidus* now seems to lie in the use of endogenous or exotic pathogens (Nankinga, 1994; Traoré, 1995).

2.9.4. Biological control using entomopathogens

Pathogens used for insect and mites control include viruses, bacteria, fungi, protozoa and nematodes. It was estimated that about 650 viruses cause disease to insects (Cloutier and Cloutier, 1992). Bacteria are frequently found in association with insects (Poinar and Thomas, 1985) but only about 100 species are insect pathogens (Cloutier and Cloutier, 1992). Among fungal pathogens, about 700 species are found to cause insect diseases (Ferron, 1978; Hall and Papierok, 1982; Burges, 1981; Cloutier and Cloutier, 1992). The number of nematodes species found in associations with insects was about 3000 (Poinar and Thomas, 1985; Cloutier and Cloutier, 1992).

Tanada and Kaya (1993) stated that the use of pathogens in the control of an insect pest has the following advantages and disadvantages:

The advantages are:

1. Specificity to target organisms or to a limited number of host species.
2. Harmless to invertebrates and plants.
3. No toxic residues.
4. Little or no environmental pollution.
5. Little or no development of resistance by the target organism.
6. No secondary pest outbreak.
7. Compatibility with many chemical pesticides, parasitoids, predators and other pathogens.

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8. Possibility of long-term control.
9. Ease of application of pathogen with conventional spray equipment.
10. Mass production capability with facultative pathogens.
11. Adaptable to genetic modification through biotechnology.

The disadvantages are:

1. Specificity only to the target organism.
2. Pathogen or its by-product(s) may be harmful to non-target organisms.
3. Strict timing of application is necessary for optimum effect.
4. Good coverage is essential for contact or ingestion of pathogen by target organism.
5. Insect death does not occur immediately after infection
6. Susceptibility to inactivation by environmental conditions.
7. Loss of virulence and pathogenicity by frequent sub-culturing.
8. Short self life and/or requirements for special handling.
9. Obligate pathogens are difficult or expensive to mass produce.
10. Uneconomical except for high-value crops.
11. Fear of pathogens by the public.
12. Risk associated with genetically engineered pathogen (host range modification, gene exchange to other organisms and genetic stability)

For the control of *C. sordidus*, entomopathogenic nematodes (Treverrow *et al.*, 1991) and entomopathogenic fungi (Batista-Filho *et al.*, 1987; Busoli et al, 1989; Nankinga, 1994; Traoré, 1995) are considered the most promising candidates.

2.9.4.1. Nematodes

The entomopathogenic nematodes used in the control of *C. sordidus* include species of *Steinernema*, *Heterorhabditis* and *Neoaplectana*. The effectiveness of these nematodes against the banana weevil was demonstrated in Australia, the Caribbean, Florida and Brazil (Figuerda, 1990; Treverrow *et al.*, 1991; Schmitt *et al.*, 1992). It was shown that entomopathogenic nematodes such as *S. carpocapsae* can cause high

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mortality (up to 85%) to the banana weevil (Treverrow, 1994). Evaluation of entomopathogenic nematodes for the control of *C. sordidus* showed significant mortality of both the banana weevil larvae already in the rhizome and the adult weevil attracted to the treatment sites (Treverrow *et al.*, 1991). The insect larvae size is a limiting factor in attempting control with steinernematid nematodes. The larvae of *C. sordidus* may need to grow appreciably before steinernematid nematodes can penetrate them. This is because steinernematid nematodes unlike heterorhabditids, can enter their hosts only through mouth, anus and spiracles (Bedding and Molyneux, 1983) and these orifices are likely to be too small in newly-hatched banana weevils. In Brazil, Schmitt *et al.*, (1992) sprayed *S. carpocapsae* suspended in water onto split pseudostem stumps which were used as a bait for the weevils. This method of application gave significantly greater control of weevil than the application of nematodes to soil around banana plants.

2.9.4.2. Fungi

The first micro-organisms found to cause diseases in insects were fungi because of their conspicuous growth on the surface of their hosts (Tanada and Kaya, 1993). Various species and strains of entomopathogenic fungi have been found and isolated from *C. sordidus* and other insects hosts. The most important are species in the genera *Beauveria*, *Metarhizium*, *Paecilomyces* and *Nomurea* (Delattre and Jean-Bart, 1978; Batista-Filho *et al.*, 1987; Busoli *et al.*, 1989; Allard and Rangi, 1991; Nankinga, 1994). Some of these entomopathogenic fungi, such as isolates of the fungi *B. bassiana* and *M. anisopliae*, have been found to be potential microbial agents against the banana weevil in Africa and elsewhere (Delattre and Jean-Bart, 1978; Mesquita, 1988; Nankinga, 1994; Traoré, 1995). Batista-Filho *et al.* (1987), infected field-collected *C. sordidus* with *B. bassiana* and *M. anisopliae* cultured on rice and beans. The *C. sordidus* mortality recorded was above 85% for *B. bassiana* cultured on the different media, while the *M. anisopliae* cultured on beans caused only 56% mortality. The same authors also tested strains of *B. bassiana* isolated from *Ligyrus* sp. (Coleoptra: Scarabaeidae) and *Diatreae saccharalis* F. (Lepidoptera: Pyralidae), and *M. anisopliae* isolated from *Ligyrus* sp. and *Deois flavopicta* Stål (Hemiptera:

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Cercopidae) against the banana weevil. The weevil mortality rate obtained was high (94.7% to 98.6%). Delattre and Jean-Bart (1978) tested *B. bassiana*, *Beauveria brongniartii* (Sacc.) Petch (= *B. tenella* (Delac) Siemi), *M. anisopliae* and *Nomuraea rileyi* (Farlow) Samson (= *Spicaria rileyi* (Farlow) Charles) under field conditions. Weevil infection ranged from 64 to 100% using a dose of 10^{11} spores/m^2. Mesquita (1988) used *B. bassiana* and *M. anisopliae* against *C. sordidus* and the weevil mortality was up to 100% and 64% respectively in 36 days for *B. bassiana* and *M. anisopliae* strains respectively. Nankinga (1994) in studies on the potential of indigenous fungal pathogens for the biological control of the banana weevil in Uganda, and Traoré (1995) in Benin reported mortalities up to 100% for *C. sordidus* adults exposed to the infective units of *B. bassiana* and *M. anisopliae*. The two authors reported that the rate of mortality of *C. sordidus* depended on isolate, conidial concentration in formulation, mode of formulation and delivery system. These studies showed the potential of *B. bassiana* and *M. anisopliae* in the microbial control of *C. sordidus*.

2.10. The entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae*

2.10.1. Taxonomy

Most fungal pathogens with good potential for development into mycopesticides belong to either the class Hyphomycetes or order Entomophthorales (Class Zygomycetes) (Moore and Prior, 1993). There are more than 700 species of entomogenous fungi in approximately 100 genera but only six species are currently registered for use in pest control (Goettel *et al.*, 1990; Robert *et al.*, 1990).

Ainsworth (1973) divided fungi into two divisions Myxomycota for plasmodial forms and Eumycota for non-plasmodial forms that are frequently mycelial. Entomopathogenic fungi are found in the division Eumycota and in the following subdivisions Mastigomycotina, Zygomycotina, Ascomycotina, Basidiomycotina and Deuteromycotina. Most entomopathogenic fungi are found in Zygomycotina, class Zygomycetes, order Entomophthorales; in Ascomycotina, Class Pyrenomycetes, order Sphaeriales; in Class Laboulbeniomycetes, order Laboulbeniales and in the

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Deuteromycotina, Class Hyphomycetes, order Moniliales (Tanada and Kaya, 1993). Selected examples of entomopathogenic fungi genera are given in Table 2.

Table 2. Outline classification of some entomopathogenic fungi.

Subdivision	Class	Order	Selected genera
Mastigomycotina	Chytridiomycetes	Blastocladiales	*Coelomomyces*
Zygomycotina	Zygomycetes	Entomophthorales	*Entomophaga, Entomophthora, Erynia, Neozygites*
Ascomycotina	Pyrenomycetes	Sphaeriales	*Cordyceps*
Basidiomycotina	Phragmobasidiomycetes	Septobasidiales	*Septobasidium*
Deuteromycotina	Hyphomycetes	Moniliales	*Beauveria, Hirsutella, Metarhizium, Nomuraea, Paecilomyces, Sorosporella, Verticillium, Fusarium*

Source Tanada and Kaya (1993)

The genera *Beauveria* and *Metarhizium* are in the class Hyphomycetes. The genus *Beauveria* has four species of which *B. bassiana* and *B. brongniartii* are the most commonly studied (McCoy *et al*., 1988). The genus *Metarhizium* has two species, *M. anisopliae* and *M. flavoviride* W. Gams & J. Rozsypal. The species *M. anisopliae* has two varieties, *M. anisopliae* var. *major* (Johnston) Tulloch and *M. anisopliae* var. *anisopliae* Tulloch (Tulloch, 1976). The genus *Metarhizium* is currently under revision.

2.10.2. Infection processes by *Beauveria* and *Metarhizium*

Entomopathogenic fungi such as species of *Beauveria* and *Metarhizium*, unlike bacteria and viruses that pass through the gut wall from contaminated food, infect their host by contact. They reach the haemocoel by penetrating the cuticle or possibly through the mouth parts and other external openings of an insect (Ferron, 1981; McCoy *et al*., 1988; Tanada and Kaya, 1993). Three phases have been recognised in the development of insect mycosis (Samson *et al*., 1988, Tanada and Kaya, 1993); (i)

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adhesion and germination of the spores to the host cuticle, (ii) penetration of the insect integument by a germ tube and (iii) development of the fungus inside the insect body, generally resulting in death of the infected host.

The adhesion of the spores onto the insect's cuticle may be a passive mechanism and involve mucilagenous material and spore surface structures (Samson *et al.*, 1988). Spore germination on the cuticle surface is affected by micro-climatic factors, especially temperature and humidity (Tanada and Kaya, 1993). For example, Ferron (1981) reported that the optimal growth temperature is 23-25°C for *Beauveria* and 27-28°C for *Metarhizium*. Penetration of hyphae through the cuticle occurs after the spores have met favourable conditions for their germination. The mode of penetration mainly depends on the property of the cuticle, it thickness, sclerotization, and the presence of antifungal and nutritional substances (Charnley, 1984). The hyphal penetration process involves both mechanical and enzymatic factors (Ferron, 1981; Samson *et al.*, 1988). The biochemical basis for pathogenesis by mycosis was discussed by McInnis in 1975 (Ferron, 1981) and Samson *et al.*(1988). Several enzymes, including lipase, protease, amylase and chitinase, are reported from *B. bassiana* that enable the hydrolysis of the protein-chitin complex of the integument (Samsinakova *et al.*, 1971; Smith *et al.*, 1981; St Leger *et al.*, 1986). The chitnase activity occurs mainly at the time of fungal growth and conidia formation (Coudron *et al.*, 1984).

Different toxins can be produced by *B. bassiana*. The toxin beauvericin, a depsipeptide (Hamill *et al.*, 1969) and other toxins in *B. bassiana* have an antibacterial effect which serves to reduce bacterial competition during the saprophytic phase of growth on the host cadaver (Ferron, 1978).

The mycosis produced by the entomopathogenic fungi imperfecti (Moniliales) *B. bassiana*, *B. brongniartii* (= *B. tenella*) and *M. anisopliae* var. *anisopliae* and var. *major* is known as white muscardine or green muscardine depending on the colour of the spores.

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2.10.3. Development of muscardine disease

Once the fungus has penetrated the integument, the muscardine caused by *Beauveria* spp. or *Metarhizium* spp. develops in the haemocoel in the presence of cellular defensive reactions of the host (Seryczynska and Bajan, 1975). Plasmotocytes surround the mycelium as pseudo-tissue or granuloma as described by Vey *et al.* (1975) in invertebrate cell culture (Ferron, 1981). *Beauveria* and *Metarhizium* produce toxins which erode the granuloma and allow blastospores to invade the haemocoel. Hyphal bodies proliferate only just before death of the host (Ferron, 1981). The role of entomogenous toxins is particularly important (Roberts, 1966; Evlakhova 1974). Several toxic cyclodepsipeptides, such as destruxins A, B, C and D and desmethyl-destruxin have been isolated from *M. anisopliae* cultures (Suzuki *et al.*, 1970, 1971). Also beauvericin has been isolated from *B. bassiana* (Hamill *et al.*, 1969) and beauvellide from *B. brongniartii* (Frappier *et al.*, 1975 cited by Ferron, 1981). The toxin production in different strains of *B. bassiana* is positively correlated with their virulence (Sikura and Bezenko, 1972, cited by Ferron, 1981). Within the same species of fungus, different strains can have very different spectra of activity (Ferron *et al.*, 1972).

Another factor that can affect muscardine infection is the quantity of spores in contact with the insects. There is a positive correlation between the number of infective spores and mortality by mycosis (Ferron, 1978; Nankinga, 1994; Traoré, 1995). For small, short-lived and soil insects, more inoculum (spores) of *B. bassiana* or *M. anisopliae* must be used, for example 10^{16} to 10^{17} conidia/ha (Müller-Kögler and Stein, 1970, cited by Ferron, 1981). With fewer spores, muscardine disease develops slowly and affects only the older larvae or adults and disturbance in fecundity and diapause of the surviving adults can occur (Faizy, 1978 cited by Ferron, 1981). In the laboratory the disease normally develops after contamination of insects either directly by spore suspensions (10^6 to 10^8 spores/ml) or by mixing soil with 10^5 to 10^8 spores/g or cm^3 for soil insects (Ferron, 1981).

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2.10.4. Saprophytic development of muscardine

The parasitic phase of fungal development ends with death of host. The mycelium then grows saprophytically through all the insect tissues in competition with the intestinal bacterial flora (Ferron, 1981). White muscardine produces oosprein, a red antibiotic pigment which colours the cadaver and curbs bacteria (Ferron, 1981). McInnes *et al.* (1974) also identified two yellow pigments in white muscardine, bassianin and tenellin.

2.10.5. Mass production of species of *Beauveria* and *Metarhizium*

Metarhizium spp. and *Beauveria* spp. are naturally-occurring in *C. sordidus* affected areas and survive as saprophytes in the absence of the hosts (Charles, 1941). This makes them suitable candidates for microbial insecticide development (Brady, 1981; Roberts, 1989; Moore and Prior, 1993). Cheap and effective mass production technologies have already been developed in some parts of the world for control of insect pests. There are, however, many more production systems in operation than are fully described in the literature. Often a successful production system is kept secret to promote commercial exploitation (Jenkins, 1995).

The ability to mass produce a pathogen becomes crucial in augmentative or inundative microbial control, where the pathogen is used as a biological insecticide (Jenkins, 1995). There are two basic methods for *in-vitro* production of entomopathogenic fungi: liquid fermentation and production on solid substrates. In general, a large surface area is needed for sporulation of fungi. Therefore grains such as rice which have a high surface area to volume ratio, are often used (Marques *et al.*, 1981; Mendonça, 1992) and have been shown to be superior to other substrates such as sweet potato, tapioca, papaya or coconut (Ibrahim and Low, 1993).

The production technique for *B. bassiana* (Samsinakova *et al.* 1971) and *B. brongniartii* (Blachère *et al.*, 1973 cited by Ferron, 1981) in submerged culture was developed a long time ago but was abandoned because of the difficulties of storing the

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infective units (spores) (Ferron, 1981). Liquid media for mass production of *B. brongniartii* blatospores in a fermentor (Blachere *et al.*, 1973, cited by Ferron, 1981) and the composition of aqueous fermentation media for the mass production of *B. bassiana* conidia (Goral, 1971, cited by Ferron, 1981) was established.

A two stage technique for mass production of *B. bassiana* conidiospores was used in the USSR (Zakharchenko *et al.*, 1963 cited by Ferron, 1981). The first stage involved the production of mycelium in a fermentor. The second phase was a surface-culture on nutrient medium in trays for sporulation. A pilot-factory in Krasnodar has produced annually 22 tons of Boverin (*B. bassiana* conidia plus an inert carrier, standardised at 6×10^9 conidia/g) (Ferron, 1981). A similar technique is used for the mass production of *M. anisopliae* (Goral and Lappa, 1973). In Brazil, the same technique was used but the trays were replaced with autoclaved polypropylene bags containing rice grains as a nutritive substrate (Guagliumi *et al.*, 1974). *M. anisopliae* is being produced on rice grains using a liquid-solid phase technique in IITA/Abomey-Calavi near Cotonou in Republic of Benin (Jenkins, 1995). Other cereals grains such as maize (Nankinga, 1994) have been suggested for the mass production of several entomopathogenic fungi imperfecti (Villacorta, 1976 cited by Ferron, 1981) but have not been used on a large-scale.

2.10.6. Formulation

One of the major limitations to the development of fungi for insect control is the lack of readily available formulation technology (Goettel and Roberts, 1992). It is through formulation that improved shelf life, persistence, efficacy, and field targeting can be achieved (Goettel and Roberts, 1992). The entomopathogenic fungi are living organisms and need to be formulated prior to application. The main aims of formulation are to provide an economical and easily useable form of the active ingredient with long shelf life, and if possible to enhance the effectiveness of the active ingredient (Auld, 1992). An active ingredient may be applied in the dry state as dust or granules or as a liquid or in the presence of liquid (Auld, 1992). Formulation

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of fungi may incorporate additives such as wetter, stickers, humectants, UV protectants, and thixotropic agents (Goettel and Roberts, 1992).

Since spores of *B. bassiana* and *M. anisopliae* can be mass-produced and dried, they can be applied as dry material or formulated in wettable powder or mixed with an inert carrier and applied as bait. *B. bassiana* was successfully formulated for grasshopper control in wheat bran baits and oil (Goettel and Roberts, 1992).

The formulation must ultimately be chosen on the basis of mode of infection, target host habitat, crop and application method.

2.10.7. Delivery systems

Fungal propagules are microscopic and most can be applied as conventional chemical insecticides. The application technique depends on the type of formulation, the insect host habitat, the insect host behaviour and host-pathogen relationship (Tanada and Kaya, 1993). Application technology involves the mechanisms of proper placement of a desirable concentration of active agent on the target site to obtain maximum effective control of the target insect (Tanada and Kaya, 1993). Microbial pesticides such as *Bacillus thuringiensis*, baculoviruses, protozoans, and fungi (e.g. *B. bassiana* and *M. anisopliae*) must be ingested or be in contact with their hosts to be effective. The short residual activity of these agents also requires that the pest consumes or comes into contact with the pathogen soon after application and this is accomplished by thorough coverage at the site of insect feeding.

Microbial pesticides are applied with equipment and technology developed for chemical pesticides such as hand-held spinning disk sprayers (i.e. Ultra low volume (ULV) sprayers) and rotary atomisers mounted on vehicles or aircaft (Tanada, 1967, Bateman, 1997). Moreover, simple techniques are also used. For example, *B. bassiana* production on rice is commercialised in Brazil. The fungus on rice grains is applied to banana stumps against adult banana weevils (Gert Roland Fischer's Company COINBIOL-GRF, Guide for the use of *B. bassiana* to control banana weevil in

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Brazil). Cubans also report the delivery of *B. bassiana* formulated in solid or in liquid form with the conventional sprayer of chemical insecticides (C.S. Gold, personal communication, 1998).

2.11. Practical and economic feasibility of biocontrol using entomopathogenic fungi

Tanada and Kaya (1993) pointed out that although chemical control of pests has been efficacious, the drawbacks such as pesticide resistance; resurgence of the target organism or emergence of secondary pests to primary pest status because of the destruction of parasitoids and predators; impact on non-target organisms, including humans; environmental pollution through the accumulation of pesticides in soil, water, and air; and residues on agricultural products and animals, have necessitated the development of more selective control methods compatible with the environment. Insect pathogens overcome many problems of chemical pesticides but are not extensively used despite their many positive features (Falcon, 1985).

The production of entomopathogens in the group of Deuteromycetes such *as Beauveria* spp. and *Metarhizium* spp. can be undertaken using cheap media (i.e. cassava products, oil palm kernel cake) available in the developing countries. The conidia of such fungi are formulated in water or oil and applied with a conventional equipment of chemical application such as ULV sprayers (Bateman, 1997). The conidia can also be produced on solid substrates and applied with the substrates which can maintain the growth of the fungi at the application site. It was reported by Ferron (1981) that many field experiments reveal the potential uses of muscardine fungi (i.e. *Beauveria* spp. and *Metarhizium* spp.). The efficacy of muscardine fungi is comparable to that of chemical insecticides, with additional advantage of noticeable long-term insect pest limitation (Ferron, 1981). Once a muscardine fungus is applied on the target pest, it can multiply and persist on the pest that it kills. The infected insect can also carry the disease from one point to another in a field, thus spreading the disease among the healthy population. For example, *B. brongniartii* caused an epizootic to *Melolontha melolontha* L. (Coleoptera: Scarabaeidae) one year after its

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application and 4 years later the muscardine appeared again in *M. melolontha* populations and caused a noticeable pest reduction (Keller, 1992).

In Africa, the development of microbial pesticides is still at an early stage. It is only in recent years that IITA/CABI has developed a microbial pesticide "Green Muscle" in which the active ingredients are conidia of *M. anisopliae*, for the control of grasshoppers and locusts (Lomer *et al.* 1997; Neethling and Dent, 1998). Even though this product was a success, the production is not economical, partly because of problems of contamination during the production processes (Cherry *et al.*, 1999). In contrast, little or no contaminant appears during the production of *B. bassiana* (author's observation). For this reason it may be possible to produce *B. bassiana* at the farmers level with little assistance. This makes *B. bassiana* a promising candidate for insect pests control at the level of resource-limited farmers. Therefore the main goal in the present study is to determine the potential and practicability of the use of *B. bassiana* in managing *C. sordidus* on plantain in West Africa.

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CHAPTER 3

BIOLOGY AND SPATIAL DISTRIBUTION OF *C. sordidus* ON PLANTAIN IN GHANA

3.1. INTRODUCTION

The banana weevil, *Cosmopolites sordidus* (Germar) is an important insect pest on plantain and banana (Afreh-Nuamah, 1993a; Gold *et al,* 1993). The female bores a small hole in the corm at the ground level and after preparing an incubation chamber deposits a single egg (Froggatt, 1925; Cuillé, 1950; Kranz *et al.*, 1977). As soon as the egg hatch the larva starts feeding by tunnelling preferably on the corm tissue. The destruction of the cortical tissue of the corm generally affects the plant nutrition and physiology (Treverrow *et al.,* 1991). Therefore the plant becomes very weak especially during the dry season and may topple (Sikora *et al.*, 1989; Swennen, 1990).

Infestation of young plants causes stunting of growth, disruption of fruiting or death. This usually occurs when infected suckers are planted or clean suckers are grown in heavily infested fields (Harris, 1947; Cuillé, 1950). In Uganda for example 60% of the suckers planted in heavily infested field may die due to weevil attack (P. Speijer, personal communication, 1997). In West and Central Africa a range of damage levels and yield losses due to *C. sordidus* have been reported. For example, in Côte d'Ivoire, yield reductions of 30 to 60% were found to be common (Vilardebo, 1973). Lescot (1988) reported yield reductions of 20 to 90% in Cameroon. In Ghana, Afreh-Nuamah (1993a) reported that one month after planting, percentage weevil infestation ranged from 0 to 82.5% depending on the origin of planting material (i.e. nursery material or ratoon material), history of land (cropped land or forest land) and cultivars. Also, Udzu (1997) reported yield reduction of 33.3% due to weevil infestation only and 86.1% yield reduction when the effect of nematodes and weevil infestation was combined

Prior to any control measure against *C. sordidus*, its biology and behaviour should be well understood. Froggatt (1925), Cuillé (1950) and Kranz *et al* (1977) reported that

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the duration of the different stages of *C. sordidus* vary widely according to season and locality.

In Ghana, recognition of *C. sordidus* as a major insect pest on plantain has occurred recently and research and information on *C. sordidus* such as its biology and the spatial distribution pattern of different stages on plantain is limited (Afreh-Nuamah, 1993b). It is based on this background that the present study was initiated. The focus was to determine the duration and the spatial distribution of the different stages of *C. sordidus* on plantain as a first step towards the evaluation of the efficacy of *B. bassiana* in controlling the weevil.

3.2. MATERIALS AND METHODS

3.2.1. Duration of different developmental stages of *C. sordidus*

The study was conducted at the Agricultural Research Station (ARS), Kade of the University of Ghana located about 120 km north west of Accra (6°09'N, 0°55'W) (Gary, 1987). The climate at ARS, Kade is characterised by two wet and two dry seasons. The major wet season extends from March to mid-July followed by a minor dry season between mid-July and early September. The second wet season extends from mid-September to the end of November while the main dry season is from November to February. The average annual rainfall is approximately 1650 mm (Obeng, 1959). The vegetation of the station is representative of the moist semi-deciduous tropical rain forest of Ghana (Taylor, 1960).

For the determination of the duration of different stages of *C. sordidus*, three different batches of adult weevils (16 females and 4 males; 9 females and 3 males; 16 females and 4 males) were collected from the same plantain field at ARS, Kade, on different days. The weevils were collected by means of pseudostem traps. The traps were 15 cm long stems, split in half (lengthways) and placed at the bases of the plantain, split side facing downwards (Ogenga-Latigo and Bakyalire, 1993). The batches of adult weevils collected were each used to infest ten pieces of corm (12 x 6 x 2 cm each) in

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separate plastic buckets in the laboratory where temperatures ranged between 22-29°C. Eggs laid on corm pieces by the first batch of weevils were collected every fourth day after infestation, over a 12-day period. For the second batch of weevils, eggs were collected, once on the fourth day and twice a day after infestation. Lastly eggs from the third batch were collected one day after infestation. Eggs were collected from the pieces of corm by scratching the outer tissue layer with a knife. The corm pieces were replaced with new ones after egg collection.

Eggs collected 4 days after infestation could not be used to determine the developmental duration of the egg stage since the date on which eggs were laid was unknown. These eggs could only be used to give the developmental duration of larval and pupal stages. Eggs collected one day after corm infestation could however be used to determine the developmental duration of the egg, larval and pupal stages. Each egg collected from the pieces of corm was transferred onto a new separate piece of corm (6 x 5 x 2 cm) through a window made with a knife. The new pieces of corm were labelled (date of collection, date of transfer and egg number). These pieces of corm were put into plastic bowls with lids. Water or moist tissue paper was put around the pieces of corm to keep them from drying (Plate 1.). Daily observations were made on each egg and the date of hatching recorded.

Two weeks after hatching of the eggs, the piece of corm was split, the larva removed and then transferred into a new piece of corm through a small hole made with a knife. The size of the holes made depended on the stage of larva and were as follow: 0.5 x 1 x 0.5 cm for two week-old larvae and 2 x 1 x 0.5 cm for three week-old to mature larvae. The hole was covered with a slender piece of split corm held in place with cello-tape. At weekly intervals the cello-tape was removed and the piece of corm was split and the larva transferred to a new piece of corm by the same technique described above. Larvae close to the pupal stage ceased to feed and could not bore holes in the pieces of corm and were thus exposed. This facilitated daily observations on mature larvae until pupal and adult stages were obtained. Dates at which stages ended were recorded. Knowing the beginning of the stage and its end, it was easy to calculate the developmental period of each stage. Data collected from the different batches of adult

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Plate 1. Plastic bowls containing pieces of corm on which *C. sordidus* eggs were incubated.

Note:
(a) Moist tissue papers were placed between the pieces of corm to prevent them from drying.
(b) During egg incubation period the plastic bowls were covered.

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weevils used were combined to establish the average duration of the developmental period of egg, larval and pupal stages and also the developmental period from egg to adult stage.

3.2.2. Spatial distribution of the different stages of *C. sordidus* on plantain

A trial was conducted at ARS, Kade, on cropped land that had been fallowed for two years and mainly colonised by *Chromolaena odorata* (L.) King & Rob. The land was cleared by slashing the weeds with a cutlass to the ground level. Three experimental plots of 15 x 15 m each were demarcated and pegged. Planting holes were dug in the experimental plots at 3 m spacing. Planting holes were 20 cm deep. The experimental plots were weeded monthly by hand using a cutlass.

A widely grown plantain cultivar in Ghana, Apantu-pa (false horn) (Schill *et al.*, 1997) was used as planting material. Sword suckers bought from farmers were freed from different stages of *C. sordidus* by paring (removal of surface tissue of the rhizome) and removing the old leaf sheath. The leaves on suckers were pruned.

Adult weevils of *C. sordidus* required for the experiment were collected from farmers' fields at Akanteng, about 45 km South East of ARS, Kade. Adult weevils were collected by hand from rotten plantain leaf sheaths and stumps and by means of pseudostem traps (Section 3.2.1). After collection, the adult weevils were kept in the laboratory at ARS, Kade in plastic buckets with lids, containing pieces of plantain corm at room temperature (25-28°C). The collected weevils were kept under laboratory conditions for about 2 weeks prior to use.

The three experimental plots were each planted with 25 plantain suckers. After 4 months, 20 adult banana weevils (4 males and 16 females) were released and confined at the base of each plant with grass mulch.

To monitor the distribution of a particular stage of *C. sordidus* within the plantain plant, one of the experimental plots was selected at random and all the 25 plants were

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uprooted. The sampling time for each stage was chosen on the basis of results obtained from the experiments on the developmental period of the different stages of *C. sordidus* (Table 4.). The levels at which the plants were sampled for the stages of *C. sordidus* were as shown in Figure 1.

i) Pseudostem (within 5 cm from the collar (ps))

ii) The rooting zone of the rhizome (ca)

iii) The remaining part of the rhizome (cb).

Egg distribution:

Five days after infestation, the number of eggs deposited by the released females of *C. sordidus* was counted at each level selected.

Larval distribution:

34 days after infestation, data were collected on:

- number of larvae
- number of pupae
- number of eggs

The number of pupae and eggs of *C. sordidus* collected during the larval distribution study was few, therefore was not analysed nor reported.

Pupal distribution:

42 days after plant infestation, data were taken on:

- number of pupae
- number of larvae
- number of eggs
- number of adults

The number of eggs, larvae and adults of *C. sordidus* collected was few during the pupal distribution study, therefore was not analysed nor reported.

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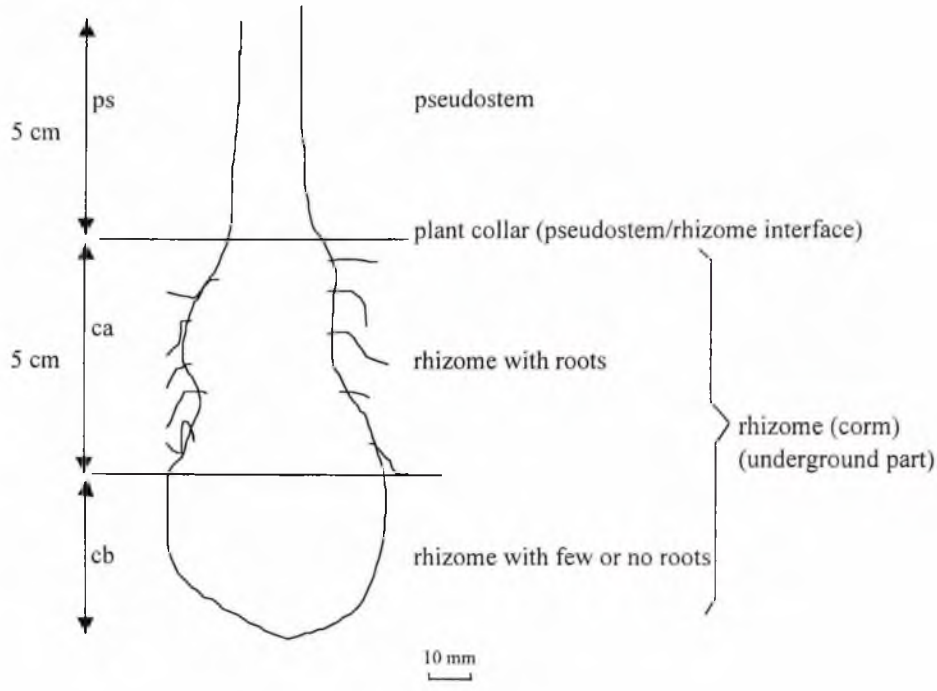

Figure 1. Levels at which uprooted plantains were sampled for weevil stages.

Note:
ps = pseudostem sampled within 5 cm from the plant collar.
ca = rooting zone of the rhizome sampled.
cb = remaining part of the rhizome sampled.

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Data were analysed using SAS version 6.12 for Windows®. Counts of egg and larvae of *C. sordidus* at the different sampling levels of the plant were subjected to Generalized Linear Models (GENMOD procedure, log linear models) analysis for comparison (SAS, 1997). The pupae of *C. sordidus* were found only at the corm level. Thus the counts of pupa were not analysed.

3.3. RESULTS

3.3.1. Duration of different developmental stages of *C. sordidus*

The number of eggs collected from adults of *C. sordidus* and percentage of viable eggs are shown in Table 3. Egg viability varied from 40 to 69% with a mean of 55 ± 9%.

The mean duration of egg incubation period, larval and pupal developmental period and the developmental period from egg to adult of *C. sordidus* was 6.3 ± 0.2, 28 ± 0.6, 7.1 ± 0.3 and 40.4 ± 0.7 days respectively (Table 3.). The duration for which most of the egg, larval and pupal stages ended are shown in Figure 2., 3 and 4 respectively.

3.3.2. Spatial distribution of different stages of *C. sordidus* on plantain

Figures 5., 6 and 7 show the percentage of eggs, larvae and pupae respectively at pseudostem and corm levels. Eggs and larvae of *C. sordidus* were found more on the underground part (corm) than on the pseudostem part of the plantain. Pupae of *C. sordidus* were not found at all at the pseudostem level. The numbers of eggs at the different sampling levels of the plant were not significantly different ($P > 0.05$) (Appendix 1.). The number of larvae of *C. sordidus* at the pseudostem level was significantly lower ($P < 0.05$) than that at the corm level ca (Table 5. and Appendix 2.). The pupae, as mentioned above, were all found at the corm level.

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Table 3. Percentage viability of eggs laid by adults of *Cosmopolites sordidus* on corm tissue under laboratory conditions.

Insect batch	Days after infestation (days)	No. of female adults	No. of eggs collected	No of eggs hatched	Percentage egg viability (%)
1	4	16	20	12	60
	4		28	16	57
	4		9	5	56
2	4	9	41	20	49
	1		19	10	53
	1		5	2	40
3	1	16	49	34	69
Mean ± SE					55 ± 9%

SE = Standard Error

Table 4. Mean duration of different developmental stages of *C. sordidus* under laboratory conditions.

Stage		Duration (days)		
	No. observed	Range	Mean	± SE
Egg	46	5-9	6.3	0.2
Larval	52	21-40	28.0	0.6
Pupal	41	3-12	7.1	0.3
Egg to adult	26	33-51	40.4	0.7

SE = Standard Error

Table 5. Parameter estimates from Generalized Linear Models (GENMOD) analysis of the number of larvae at three levels on growing plantain (Figure 1.).

Parameter	df	Estimate	SE	chi-square	P > Chi
Level ca	1	1.6582	0.5455	9.2391	0.0024
Level cb	1	1.1787	0.5718	4.2494	0.0393
Level ps	0	0.0000	0.0000		

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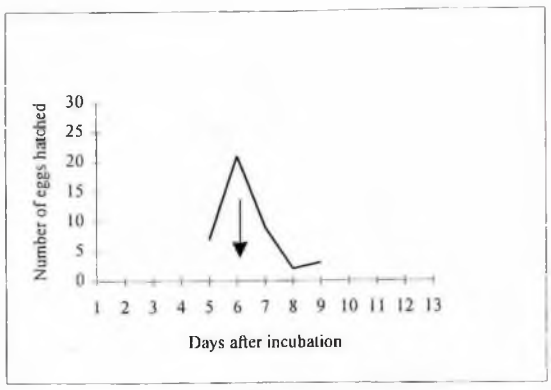

Figure 2. Duration of egg of *C. sordidus* incubation on corm pieces in the laboratory.
Note: The arrow shows the period at which a great number of the eggs hatched.

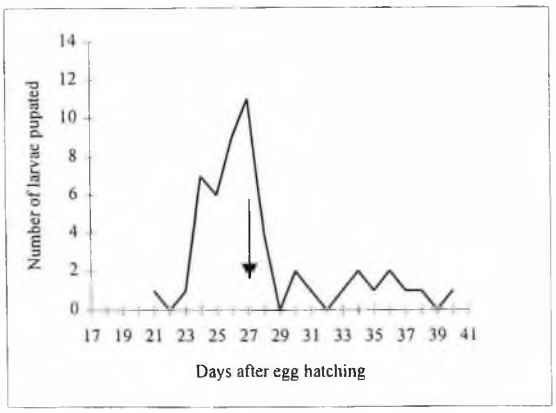

Figure 3. Duration of larval stage of *C. sordidus* in corm pieces in the laboratory.
Note: The arrow shows the period at which most of the larvae pupated.

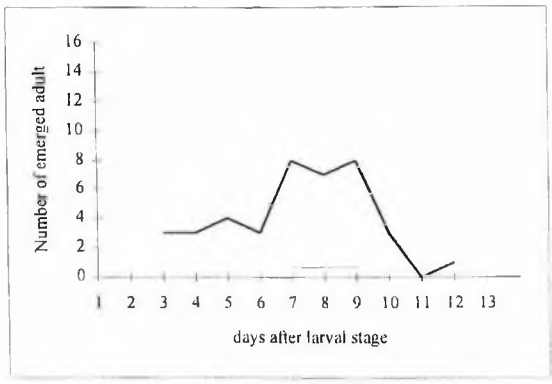

Figure 4. Duration of pupal stage of *C. sordidus* in corm pieces in the laboratory.
Note: The straight line shows the range of time (days) where most of the pupal stages lasted.

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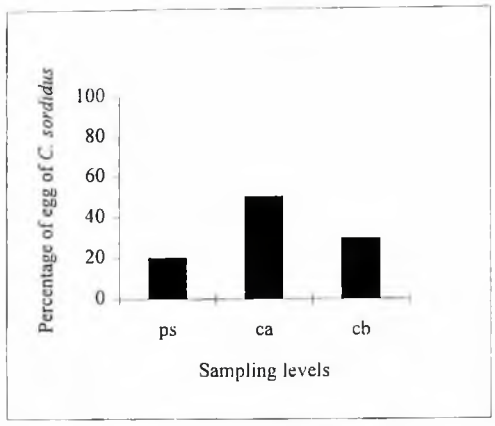

Figure 5. Percentage of eggs of *C. sordidus* at three sampling levels (Figure 1.) on plantain.

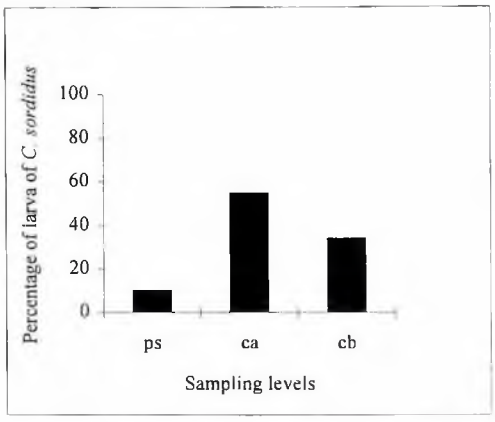

Figure 6. Percentage of larvae of *C. sordidus* at three sampling levels (Figure 1.) on plantain.

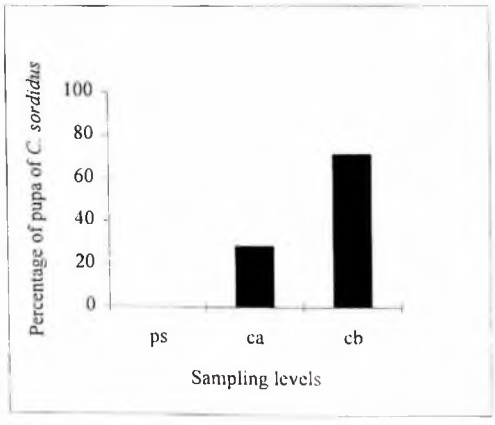

Figure 7. Percentage of pupae of *C. sordidus* at three sampling levels (Figure 1.) on plantain.

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3.4. DISCUSSION

The duration of developmental periods for egg and pupa were within the ranges reported by Cuillé (1950), Woodruff (1969), Bakyalire and Ogenga-Latigo (1992), Hill (1983), Afreh-Nuamah (1993b) and Traoré (1995). However, the range of larval stage duration differed from that observed by Afreh-Nuamah (1993b) who conducted his experiment where the present experiment was conducted. The materials and methods used, environmental conditions (i.e. temperature) (Traoré, 1995), seasonal variation (Cuillé, 1950) and even the biotype of *C. sordidus* may account for such differences.

The results from the present work can be used in laboratory and field trials to guide data collection on the different stages since the experiment was conducted within the temperature range prevailing in the field. Similarly, the feeding material used to follow the developmental period of the larva was the same as in nature but one variation might be that cut corms are more palatable as they start rotten to larvae than growing ones.

The spatial distribution of eggs observed in this study agree with that reported by Froggatt (1925). The adults of *C. sordidus* deposited their eggs both on the pseudostem and on the corm of plantain but more eggs were laid on the rhizome. The results obtained for the present study contrast with that of Abera *et al.* (1997) who found more eggs on the pseudostem than on the corm. One of the reasons for such dissimilarity may be the plant nature (i.e. morphology) since the trials were carried out on two different *Musa* spp. (banana and plantain) and another reason may be differences in soil type.

The spatial distribution of the larvae of *C. sordidus* (Figure 6.) followed that reported by Knowles (1918), Moznette (1920), Frogatt (1925), Cuillé (1950) and Abera *et al* (1997). Cuillé (1950) observed that on the growing banana, tunnels of larvae of *C. sordidus* were downward in the rhizome and the grub rarely occurred in the

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pseudostem because the high moisture content of the pseudostem is not favourable. This observation is supported by that of Abera *et al* (1997) who found that even though more eggs were laid in the pseudostem, more than 80% of the banana weevil larvae were located within the corm. Cuillé (1950), on the other hand reported that larvae were often found in fallen dehydrated pseudostems. Thus the larval habitat depends on the state of the plant.

In the present study, pupae of *C. sordidus* were found only in the rhizome. This result confirmed that of Frogatt (1925) who reported that the pupae of *C. sordidus* were found only in the underground (corm) part of the banana plant. The plant site where the larvae of *C. sordidus* pupate is the site of emergence of the adult. All the stages, except the pupal stage, of *C. sordidus* occurred both on the pseudostem and rhizome of growing plantain.

The knowledge of location of *C. sordidus* stages within the plantain is a useful tool for efficient and economical application of *B. bassiana*. In this study eggs and larvae of *C. sordidus* were found both in the corm and pseudostem of plantain while the pupae were found only in the corm. Thus the delivery of *B. bassiana* against the egg or larval stage must be directed to both the pseudostem and corm of plantain while that against the pupal and adult stage must be towards the corm from the plant collar or on the soil around the plant.

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CHAPTER 4

SELECTION OF STRAIN OF *B. bassiana* FOR THE CONTROL OF *C. sordidus*

4.1. INTRODUCTION

An important step in the development of a mycoinsecticide for the control of an insect pest is the selection of the most effective strain (s) with:

i) a high level of virulence to the specific target pest

ii) some appropriate production features including
   - high productivity,
   - optimum utilisation of available substrate
   - genetic stability, and

iii) a good field performance with respect to insect mortality and persistence in the ecosystem (Prior, 1992; Moore and Prior, 1993; Jenkins and Goettel, 1997b).

The strains of a given fungal pathogen species can exhibit wide variability in their pathogenicity and virulence against an insect host (Prior *et al.*, 1988; Pena *et al.*, 1993; Tanada and Kaya, 1993; Nankinga, 1994). Factors that affect the pathogenicity and virulence of fungal pathogens include source of the strain (Fargue, 1972; Soper and MacLeod, 1981; Prior *et al.*, 1988; Moore and Prior, 1993), and method of culturing (Soper and Ward, 1981; Batista-Filho *et al.*, 1987). In general, strains of a species from a specific host are more pathogenic for that host than those from other hosts (Tanada and Kaya, 1993). Fungi are also known to lose their virulence after being repeatedly cultured on artificial media (Jenkins, 1995).

In addition to the biological characteristics of the fungal agent, the choice of delivery technique of a fungal pathogen in relation to the behaviour and ecology of the target pest also plays an important role in the development of a mycoinsecticide. The banana weevil is cryptic and spends the greater part of its life cycle hiding within the plantain. Because of this cryptic habit the weevil can not be sprayed with a mycopesticide.

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Therefore, there is a need to find a suitable delivery material for the selection or evaluation of a mycopesticide against the banana weevil. Cut pseudostems or cut corms have been used to trap the banana adult weevils. These traps can be used either in combination with chemical insecticides (Froggatt, 1923; Ogenga-Latigo and Mazanza, 1996) or with *B. bassiana* (Nankinga, 1994; Traoré, 1995; Ogenga-Latigo and Mazanza, 1996) for the weevil population reduction.

Apart from being virulent, the pathogen should also have the potential for mass production (Goettel and Roberts, 1992). Tests on the virulence of a fungal pathogen can be easily conducted in the laboratory using a small amount of conidia cultured on artificial media in Petri dishes or glass bottles. However, for field trials where a large surface area is treated, the production of infective units (i.e. conidia) on artificial media is not economically sound. The ability to mass-produce a pathogen on cheap and available media becomes crucial in augmentative or inundative microbial control, where the pathogen is used as a biological insecticide. Therefore the selection of a fungal pathogen must take into consideration its potential for mass production. Solid substrates have commonly been used for the production of entomopathogenic fungi (e.g. *Beauveria* spp. and *Metarhizium* spp.) (Ferron; 1981; Jenkins and Goettel, 1997b). Rice has been shown to be superior to other substrates such as sweet potato, tapioca, papaya or coconut (Ibrahim and Low, 1993), and has been commonly used for the production of entomopathogenic fungi on a large scale.

To achieve effective control of an insect pest, it is necessary to select strains, which combine the best possible characteristics (i.e. virulent and easy to mass-produce) for killing the target (Prior, 1992). On this basis, the present study of selection of the best strain for further research, aims at determining:

(i) The virulence of imported strains of *B. bassiana* to *C. sordidus* using pseudostem traps or cut corms as delivery materials

(ii) The potential of *B. bassiana* strains for mass production using rice grains as a solid substrate

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4.2. MATERIALS AND METHODS

4.2.1. Strains of *B. bassiana* used in experimental work

Strains of *B. bassiana* used in the present work are shown in Table 6. It was reported by Traoré (1995) that the strain I94-907 (Table 6.) was previously isolated from *Cylas* spp. (Coleoptera: Brentidae). It was imported from CABI Bioscience (ex International Institute for Biological Control in Ascot) to Benin and used against *C. sordidus* (Traoré, 1995). The infected banana weevils were stored in a refrigerator for one year at temperatures below 10°C. The strain ARSEF2624 was imported from USA on Sabouraud Dextrose Agar (SDA) in 9 cm Petri dishes. The strain IMI330194 was imported from the International Mycological Institute (IMI) on Potato Dextrose Agar (PDA) in 25 ml Universal bottles.

Table 6. *B. bassiana* strains used during trials.

Strain codes	Species	Host	Origin
[1]ARSEF2624	*B. bassiana*	*Cosmopolites sordidus* (Coleoptera: Curculionidae)	unknown
[2]I94-907	*B. bassiana*	*Cylas* spp. (Coleoptera: Brentidae)	Uganda
[3]IMI330194	*B. bassiana*	*Hypothenemus hampei* (Coleoptera: Scolytidae)	Kenya

[1] collection of Agricultural Research Service Collection of Entomopathogenic Fungi (ARSEF)

[2] collection of CABI Bioscience Silwood Park, Ascot, England

[3] Collection of International Mycological Institute (IMI)

The three strains of *B. bassiana* (Table 6.) tested for their virulence during the trials were received at different times of the study. Therefore, subsequent experiments did not include all the strains simultaneously. Also if a strain exhibited low virulence during an experiment it was excluded from successive experiments. The concentration of conidia, and the number of replications of treatments in the different experiments

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were affected by the quantity of formulation available at the time the strains were to be tested. But the doses of conidia applied were within the range of that found to induce mortality to *C. sordidus* (Nankinga,1994; Traoré, 1995).

4.2.2. Comparative virulence of the strains ARSEF2624 and I94-907 to adults of *C. sordidus*

The strains ARSEF2624 and I94-907 were cultured on amended SDA. The medium SDA was prepared following manufacturer's instructions (Merk/BDH). A quantity of 65g of SDA powder was dissolved in 1 litre of water by heating in a boiling water bath. The Agar suspensions were then amended with 10 ml 95% alcohol (ethanol) plus antibiotic Chloramphenicol (0.05 g in 10 ml 95% alcohol) per one litre of media and autoclaved for 15 min at 121°C and 120 kPa (Jenkins and Goettel, 1997a). A quantity of 5 ml, 7 ml and 100 ml of the media were poured into 9 cm Petri dishes, 25 ml Universal glass bottles, and 700 ml square sided Whisky glass bottles (slopes) respectively. Media pouring was done in a sterile air flow cabinet to avoid contamination. All aseptic techniques required for fungal isolation and culturing were also applied.

Conidia of the strain I94-907 were picked from the fungus cultured on dead adults in the laboratory while conidia of the strain ARSEF2624 were taken from imported cultures in Petri-dishes (Section 4.2.1) with a sterile bacteriological needle. The conidia of the strains on the tip of the needle were each streaked onto SDA in Petri dishes (Jenkins and Goettel, 1997a). The Petri dishes were stored in cupboards at 25-28°C for 14 days. Conidia from clean cultures were sub-cultured onto SDA slopes in 25 ml universal glass bottles for 21 days at 25-28°C. Conidia from the glass bottles were used to inoculate the amended SDA in the 700 ml square sided glass bottles. The bottles were incubated at 25-28°C in the laboratory for 21 days to allow fungal growth and conidial production. About 50 ml of sterile distilled water (SDW) plus 0.05% Tween 80 was then poured into each bottle. The bottles were hand-shaken and the resulting conidial suspension sieved (mesh 106 µm). The conidial concentration and

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viability of the strains ARSEF2624 and I94-907 in the suspensions were determined for the virulence test as follows:

i) Conidial concentration

A conidial suspension was serially diluted to get a light whitish suspension for easy counting of conidia: 1 ml of the original conidial suspension was added to 9 ml SDW plus 0.05% Tween 80 and 1 ml of the diluted suspension was then re-diluted in 9 ml SDW plus 0.05% Tween 80. The concentration of conidia in the suspension was determined using an improved Neubauer Haemacytometer and microscope (magnification x200). Preparation of the haemacytometer for counting and counting techniques was the same as that used in "Lutte Biologique contre les Locustes et Sauteriaux (LUBILOSA)" project at IITA (Jenkins and Goettel, 1997a; Appendix 3.)

ii) Conidial viability

Diluted suspensions prepared above with a low concentration of conidia (approximately 10^6 spores/ml) were used. Three droplets (120 µl) of the conidial suspension from a Pasteur pipette were spread on plated amended SDA in Petri dishes. Three replications were made. The Petri dishes were incubated at room temperature (25-28°C) for 18 h. One hundred conidia were viewed at random in each Petri dish using a microscope (magnification x200). The percentage of germinated conidia was calculated for each Petri dish. The mean for the three replicates was used as an indicator of conidial viability.

iii) Virulence determination

In order to test the virulence of the strains, the conidial concentration in the inoculum was adjusted to 10^8 conidia/ml for both strains. Pseudostem traps (cut pseudostem, 15 cm in length and split lengthways into two) and rounded pieces of corm (diameter 16 cm, 2 cm thick) were used as delivery materials for the fungal inoculum (conidia plus SDW plus 0.05 % Tween 80) and the control (SDW plus 0.05 % Tween 80) in plastic buckets with lids containing 800 ml soil each. Three batches of four pseudostem traps

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and three batches of five pieces of corm were prepared for each of the two fungal inocula and the control. Delivery materials in a specific batch were soaked individually in 30 ml of one of the fungal inocula or in 30 ml of SDW plus 0.05% Tween 80 for 30 min. The remaining inoculum (approximately 25 ml) was poured onto the soil covering the surface area on which the delivery material was deposited. A total of 20 adult banana weevils (10 males and 10 females) were introduced into each plastic bucket. After 5 days, all the weevils were removed with a forceps from the traps and soil in the plastic buckets and kept in Petri dishes with fresh pieces of corm (3 x 3 x 2 cm) at room temperature (25-28°C). Daily observations were made on the insects for 21 days. Dead insects were collected from the Petri dishes and counted. To determine external growth of *B. bassiana*, the dead insects were washed with SDW, disinfected with 70% alcohol, rinsed with SDW and were finally incubated in Petri dishes (85 mm diameter and 15 mm deep) lined with sterile moist tissue paper in the laboratory at temperatures of 25-28°C. The incubated dead insects were checked daily for mycelium or sporulation of *B. bassiana*. Percentage cumulative mortality and percentage dead adults with signs of mycosis were calculated after 7, 14 and 21 days. The calculated percentages were angular transformed (Gomez and Gomez, 1984) and subjected to analysis of variance (ANOVA) using the Satistical Analysis System (SAS) version 6.12 for Windows® (SAS, 1997). Transformed mean percentage dead weevils and transformed mean percentage dead weevil with signs of mycosis were each compared using Tukey's test in SAS. Detransformed means were presented in Tables (Section 4.3.1.).

4.2.3. Comparative potential for mass production and virulence of the strains I94-907 and IMI330194 of *B. bassiana* to *C. sordidus*

The strain ARSEF2624 showed low virulence compared to the strain I94-907 (Table 7.). Therefore it was excluded from further experiments. As the selected strain I94-907, was being mass produced, the imported strain, IMI330194, was received so comparative studies were carried out on both strains for potential of mass production and virulence.

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4.2.3.1. Comparative potential for mass production of the strains I94-907 and IMI330194 of *B. bassiana*

Unlike the previous experiment where conidia produced on amended SDA were used, in this experiment, conidial powder of the two strains was used in the comparative studies described below. The production of the conidial powder involved liquid and solid phases using the modified method of Jenkins (1996). The liquid phase was to provide active growing mycelium and blastospores while the solid phase was to provide dry aerial conidia. The different phases in the procedure were as follows:

i) Liquid phase

The liquid medium was prepared using waste brewers yeast, collected from the local brewery (Brasserie: La Béninoise de Cotonou). The yeast was obtained as a slurry and processed by first boiling in a bowl filled at half of its volume on a gas cooker for approximately 30 min. The yeast solid was then separated by filtering off the excess water through a tissue paper. The yeast solid thus obtained was heat dried in an oven at 80°C for 1 to 2 days. The yeast was then cooled at room temperature of 26°C and ground into powder using an ordinary corn mill machine. The resulting powder was stored at room temperature in plastic bottles with lids until needed for use. Yeast prepared in this manner was good for use up to 6 months after preparation without a reduction in the yield of conidia from the production system (Jenkins, 1995, 1996).

The liquid medium was prepared by re-suspending yeast powder in tap water (20g l^{-1}). The suspension was heated to boiling point to dissolve the yeast powder. The yeast solution was then homogenised in a commercial Waring blender set at high speed for 1 min to further break up the yeast cells. Locally purchased sucrose (sugar) was then added to the hot broth (20g l^{-1}), and 75 ml of the medium was then distributed into 250 ml Erlenmeyer flasks. The flasks were plugged with polyurethane bungs, covered with aluminium foil and autoclaved for 30 min at 121°C and 120 kPa. The liquid medium in the flasks was allowed to cool and was then inoculated with 1 ml of *B. bassiana* conidia suspension (approximately 10^7 conidia/ml) extracted from amended

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PDA. The preparation, inoculation of the amended PDA and conidial extraction for inoculation were as described for amended SDA in Section 4.2.2.

The liquid media in the flasks inoculated with conidia of the strains I94-907 or IMI330194 were incubated on a rotary shaker (Plate 2.) at 150 rpm for 3 days at 28-30°C in the laboratory. The liquid culture in each flask was diluted by 50% with sterilised tap water and used for inoculation of solid medium (rice grains) in the solid phase.

ii) Solid phase

Rice purchased at the local market was washed, drained and distributed into locally made polypropylene carrier bags (250 g per bag). The bags containing rice were autoclaved at 121°C and 120 kPa for 45 min. Once cooled, 37.5 ml of the diluted liquid culture was added to each bag and mixed (Plate 3.) The inoculated bags were placed in plastic basins which had been previously washed with water plus sodium hypochlorite (Eau de Javel 0.1%) before use. The basins were prepared to allow aeration by making four holes of 27 mm diameter each around their circumference. The holes were closed with sterilised polyurethane bungs. The bags were not sealed within the basins. The basins were initially closed with lids and stacked (Plate 4a.) for at least 4 days after which time, the lids were replaced with wooden supports and the bungs were removed to allow air drying of the conidia and substrate (Plates 4b. and 4c.) for 8 to 12 days. The room temperature fluctuated between 20 to 23°C during the incubation and drying period. The conidia were sieved from the solid substrate using a sieve (mesh of 106 μm) (Plate 5.) The resulting conidial powder (Plate 6.) was dried for 3 days in a dehumidifier (Plate 7.) containing non-indicating silica gel.

In order to compare potential of the two strains to produce conidia, data were taken on number of conidia per gram of rice, weight of conidial powder produced per kilogram of rice, number of conidia per gram of powder, and viability of conidia.

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100 mm

Plate 2. Incubation of *B. bassiana* conidia in liquid medium in Erlenmeyer flasks (a) placed on a rotary shaker (b) for production of hyphae and blastospores.

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100 mm

Plate 3. Inoculation of autoclaved rice grains in polypropylene bag with liquid medium containing *B. bassiana* hyphae and blastospores.

Note: Author holding polypropylene bag containing rice grains being inoculating with liquid medium of *B. bassiana* hyphae and blastospores (arrow) in Erlenmeyer flask.

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Plate 4. Incubation and air drying of rice grains inoculated with *B. bassiana* in plastic bowls.

Note: (a) Closed bowls contained the inoculated rice grains for incubation.
(b) Lids of bowls removed to facilitate air drying.
(c) Close-up view of air dried inoculated rice grains viewed from above.

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Plate 5. Extraction of conidial powder of *B. bassiana* from rice grains.

Note:
Arrow indicating the pouring of sporulated rice grains into a sieve having a container underneath for conidial powder collection with the help of an assistant.

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Plate 6. Conidial powder of the *B. bassiana* strain IMI330194 obtained from rice grains in Petri dish.

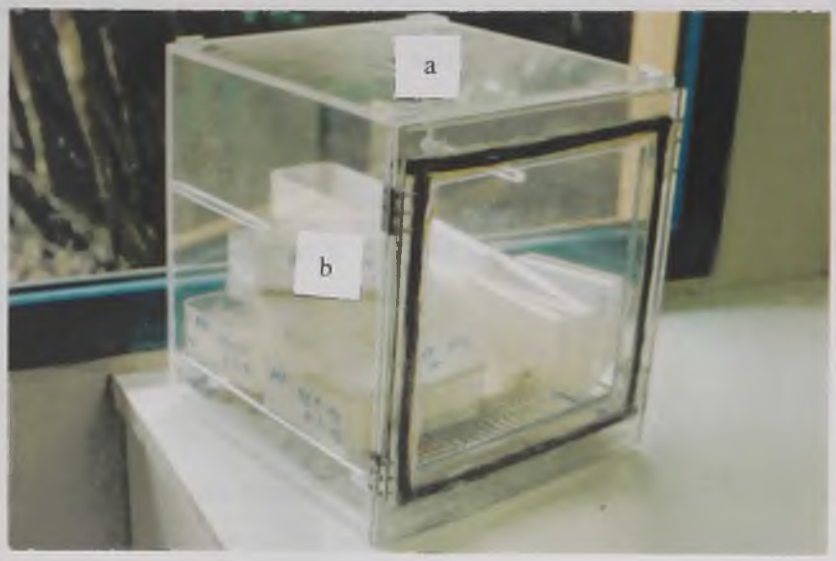

Plate 7. A dehumidifier (a) containing silica gel for drying conidial powder placed in plastic container (b).

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- Determination of the number of conidia per gram of rice

After the inoculated rice was air dried, 1 g was placed in a 25 ml glass bottle and 10 ml of SDW plus 0.05% Tween 80 was added. The bottle was hand-shaken and the conidial solution was sieved (mesh of 106 µm) sonicated. A serial dilution was made and the spore concentration was determined using a Neubauer haemacytometer. The technique of preparation of the haemacytometer for counting was the same as that used in "Lutte Biologique contre les Locustes et Sauteriaux (LUBILOSA)" project at IITA (Jenkins and Goettel, 1997a, See Appendix 3.). The procedure for counting was repeated three times for each strains. The counts of conidia were subjected to log transformation and ANOVA for comparison. Detransformed means were presented in Section 4.3.2.

- Determination of the weight of conidial powder per kilogram of rice

The conidial powder produced on 250g of inoculated and incubated rice was weighed. The weight of conidial powder from four plastic bowls gave the weight of conidial powder per kg of rice. The mean weight from four sets of four plastic bowls was used to express the conidial powder weight per kg of rice. The procedure was replicated four times for each strain. The conidial weights were square root transformed (Gomez and Gomez, 1984) and subjected to ANOVA (SAS, 1990). Detransformed means were presented in Section 4.3.2.

- Determination of the number of conidia per gram of powder

A weight of 0.02 g conidial powder for easy dilution and conidial counting was placed in each of three 25 ml glass bottles. In each bottle, 10 ml of SDW plus 0.05% Tween 80 was mixed with the conidial powder. The mixture was sieved, then sonicated for less than 1 min to break up conidial chains and reduce conidial clumping. Serial dilution was done to facilitate counting of conidia and a Neubauer haemacytometer was used to determine the conidial concentration in 1 ml conidial suspension (See

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Appendix 3.). The number of conidia per gram of powder was determined by extrapolation. The procedure was replicated four times for each strain. The number of conidia were log transformed and subjected to ANOVA (SAS, 1990) for comparison. Detransformed means were presented in Section 4.3.2.

- Determination of the viability of conidia

The conidial viability of the strain was determined on PDA in three Petri dishes as described in Section 4.2.2. The conidial suspension was diluted up to 10^6 conidia/ml. A quantity of 120 µl of the suspension were deposited on each of three plates using a Pasteur pipette. The Petri dishes were incubated at 25-28°C for 18 h. Approximately 100 conidia per Petri dishes were examined and the number of germinated and non-germinated conidia were counted. The percentages of germinated conidia in the Petri dishes were calculated. The percentage viability of conidia were subjected to square root transformation and ANOVA (SAS, 1990) for comparison. Detransformed means were presented in Section 4.3.2.

4.2.3.2. Comparative virulence of the strains I94-907 and IMI330194 of *B. bassiana* to *C. sordidus*.

Studies were carried out to compare the virulence of *B. bassiana* strains I94-907 and IMI330194 to *C. sordidus* adults, eggs and larvae using dry aerial conidia of the two strains produced in Section 4.2.3.1.

In order to test the virulence of the two strains on adults of *C. sordidus*, fungal inoculum was prepared by mixing the conidial powder of the strains with SDW plus 0.05% Tween 80. Conidial concentration and viability in the inoculum was determined as described in Section 4.2.2. and concentration adjusted to 4 x 10^9 conidia per ml for both strains.

Pseudostem traps were prepared, treated with the fungal inoculum as described in Section 4.2.2.and control (SDW plus 0.05% Tween 80).

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The quantity of fungal inoculum and control used for the pseudostem traps treatment, number of insects released, observations, frequency of data collection and analysis were the same as in Section 4.2.2.

In order to determine the virulence of the strains I94-907 and IMI330194 on eggs of *C. sordidus*, two experiments were conducted separately using the strains I94-907 and IMI330194 in turn. It was not possible to simultaneously carry out the experiments with the two strains and control because of difficulties to obtain enough eggs of the same age. Trials were therefore, carried out sequentially to compare the effect of the inoculum of each strain with the control on eggs of *C. sordidus*.

In the first experiment, 16 females and four males of *C. sordidus* were confined in a plastic bucket (25 litres volume) containing five pieces of corm for oviposition and feeding. The pieces of corm were removed one day after infestation and replaced with new ones. The surface of the removed corm was scratched with a small knife to collect *C. sordidus* eggs. Eggs were collected from the same batch of banana weevils on five consecutive days. On each date of egg collection, two rounded pieces of corm (16 cm diameter and 2 cm thick) were made. One of the pieces was soaked in 20 ml of a water formulation of the strain I94-907 (10^9 conidia ml^{-1}) for 30 min. The second piece of corm was soaked in SDW plus 0.05% Tween 80 (control) for 30 min. The collected eggs were equally distributed on the treated pieces of corm through punctures made with forceps.

For the second experiment, the methodology of egg collection and egg infection were the same as in the first experiment but the strain of *B. bassiana* used was IMI330194.

In both experiments daily observations were made on the eggs for 10 days after placement on treated corn and the number of unhatched eggs was counted. The numbers of eggs covered with fungal mycelium or conidia were also recorded. The percentages of non-hatched eggs and those showing signs of mycosis were then

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calculated, angular transformed and subjected to the ANOVA procedure in SAS. Detransformed means were presented in figures (Section 4.3.3.).

Two experiments were conducted separately to determine the virulence of the strains I94-907 and IMI330194 to *C. sordidus* larvae using the two strains in turn. The experiments could not include the two strains simultaneously because it was difficult to rear enough larvae of the same ages due to the low rate of oviposition of the adult weevils and high rate of larvae mortality. Thus trials were carried out to compare the effect of the inoculum of each strain with the control on the larvae.

Beauveria bassiana strain I94-907 was used in the first experiment. Larvae were obtained from eggs laid by field collected adult weevils and reared on corm pieces placed in plastic buckets at room temperatures (25-28°C). when larvae were two weeks old, each larva was introduced into a small piece of corm (6 x 5 x 2 cm) through a window made with a knife. One hour later, after the larva had bored and had gone into the piece of corm, 20 ml water formulation of *B. bassiana* strain I94-907 containing 10^9 conidia/ml or SDW plus 0.05% Tween 80 (control) were poured on the piece of corm at the window side. The pieces of corm were placed in a plastic box with lid. One day after inoculation, the remaining solution not absorbed by the corm was decanted from the box. A total of 25 larvae were used for each treatment. One week after treatment, the pieces of corm were split and the dead larvae recorded. They were then surface sterilised with 70% alcohol, washed with SDW and were incubated on wet tissue paper in Petri dishes to determine external growth of *B. bassiana.* The surviving larvae or pupae were transferred into new pieces of corm and observations made on them two weeks later. Data were collected on number of dead larvae, pupae and emerged adults. The proportion of dead larvae was analysed using Generalized Linear Models (GENMOD, logit model) procedure in SAS version 6.12 for Windows®. Detransformed means are presented in figures (Section 4.3.3.).

B. bassiana strain IMI330194 was used for the second experiment. The methodology for testing the virulence of IMI330194 to larvae of *C. sordidus* was the same as in the

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first experiment except that the number of larvae used was 20 per treatment because of shortage of larvae.

4.3. RESULTS

4.3.1. Comparative virulence of the strain ARSEF2624 and I94-907 of *B. bassiana* to adults of *C. sordidus*

Daily percentage cumulative mortality of *C. sordidus* adults exposed to pseudostem traps or cut corms treated with the strain I94-907 or ARSEF2624 inoculum was higher than that of adult weevils exposed to pseudostem traps or cut corms treated with control (Figure 8a.). Analysis showed that there was a significant effect of fungal strain on percentage cumulative mortality of adult weevils (Table 7.; Appendix 5.) with the strain I94-907 giving significantly higher mortality (22.3-27.1%) than the strain ARSEF2624 (4.9%) and the control (1.1%) at days 14 and 21 after exposure. This significant difference did not show between the two fungal strains 7 days after exposure. The percentage cumulative mortality caused by the strain ARSEF2624 (2.5-4.9%) was not significantly different at the 5% level compared with that of the control (0.4-1.1%) over the study period (Table 7.).

Daily percentage cumulative dead weevils with signs of mycosis after exposure to pseudostem traps or cut corms treated with I94-907 inoculum was higher compared with percentage cumulative dead weevils with signs of mycosis after exposure to pseudostem traps or cut corms treated with ARSE2624 inoculum or control (Figure 8b.). Statistical analysis showed that the percentage cumulative dead adults showing signs of mycosis at death (0.0%) after exposure to the strain ARSEF2624 or control was significantly lower over the study period compared with those exposed to the strain I94-907 (1.2-18.4%) (Table 8.; Appendix 6.).

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Table 7. Mean percentage cumulative mortality of *C. sordidus* adults 7, 14, 21 days after exposure to pseudostem traps and pieces of corm treated with inoculum of *B. bassiana* strains ARSEF2624 or I94-907.

Strain	Days after exposure		
	7	14	21
I94-907	6.5a	22.3a	27.1a
ARSEF2624	2.5ab	4.9b	4.9b
Control	0.4b	1.1b	1.1b

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test).

Table 8. Mean percentage cumulative dead weevil with signs of mycosis 7, 14 and 21 days after exposure to pseudostem traps and pieces of corm treated with inoculum of *B. bassiana* strains ARSEF2624 or I94-907.

Strain	Days after exposure		
	7	14	21
I94-907	1.2a	17.6a	18.8a
ARSEF2624	0.0b	0.0b	0.0b
Control	0.0b	0.0b	0.0b

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test).

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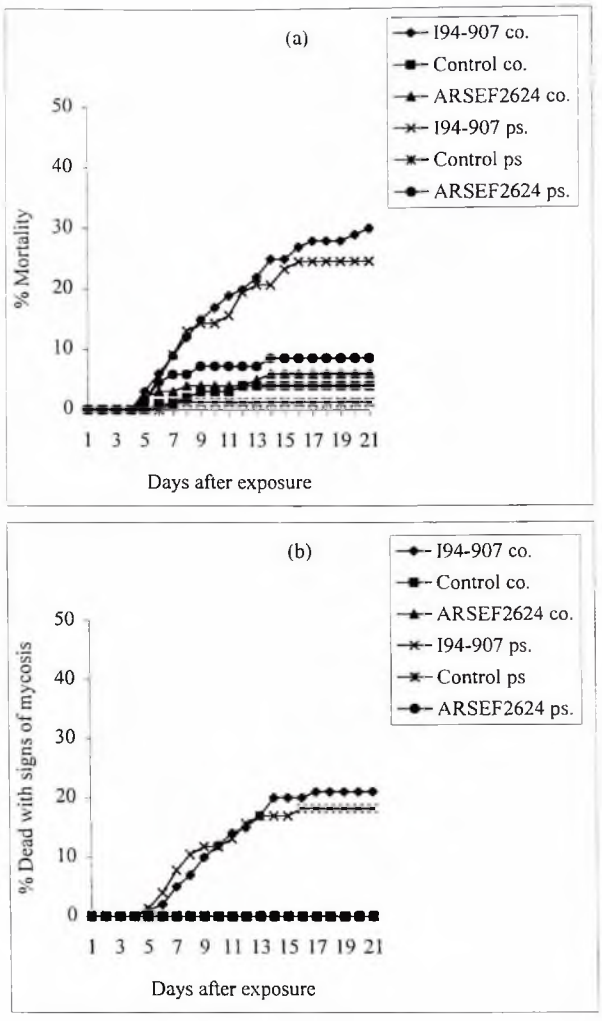

Key: co = corm ps = pseudostem

Figure 8. Percentage mortality of *C. sordidus* adults (a) and percentage dead adults with signs of mycosis (b) after exposure to pseudostem traps and pieces of corm treated with *B. bassiana* inoculum

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4.3.2. Comparative potential for mass production of the strains I94-907 and IMI330194 of *B. bassiana*

The number of conidia (6 x 10^8/g rice) produced by the strain IMI330194 per gram of rice grains was higher than that (5 x 10^8/g rice) produced by the strain I94-907 (Figure 9.) but this difference was not significant ($P > 0.05$; Appendix 7.).

Mean weight of conidial powder of IMI330194 (10.1g/kg rice) extracted from the rice grains was higher than the mean weight of conidial powder of I94-907 (7.6 g/kg rice) extracted from the same substrate (Figure 10.). However, this difference was not significant ($P > 0.05$; Appendix 8.).

The mean number of conidia of IMI330194 per gram of conidial powder (2.4 x 10^{10} conidia) was higher compared with that of I94-907 (2.0 x 10^{10} conidia) (Figure 11.). However this difference was not significant ($P > 0.05$, see Appendix 9.).

The viability of conidia of the strain IMI330194 (>95%) either on rice grain or extracted powder from rice grain was significantly higher at the 5% level compared to that of the conidia of the strain I94-907 (<90%) (Figures 12. and 13; Appendices 10. and 11.).

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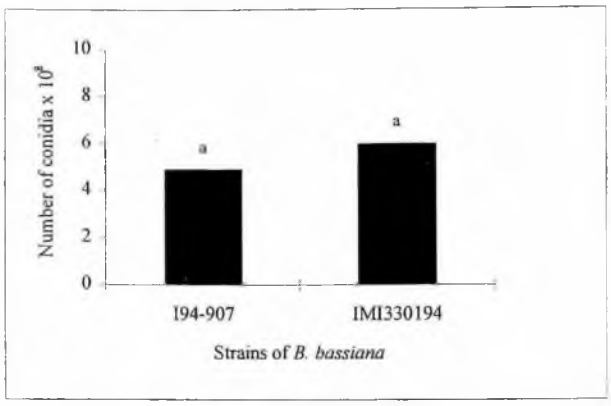

Figure 9. Mean number of conidia of *B. bassiana* strains per gram of rice grains.

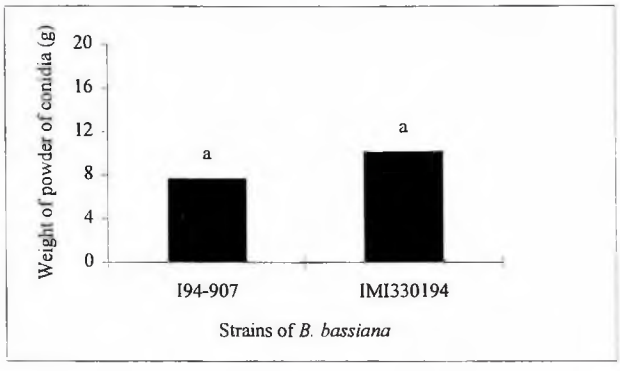

Figure 10. Mean weight of conidial powder of *B. bassiana* strains per kilogramme of rice grains.

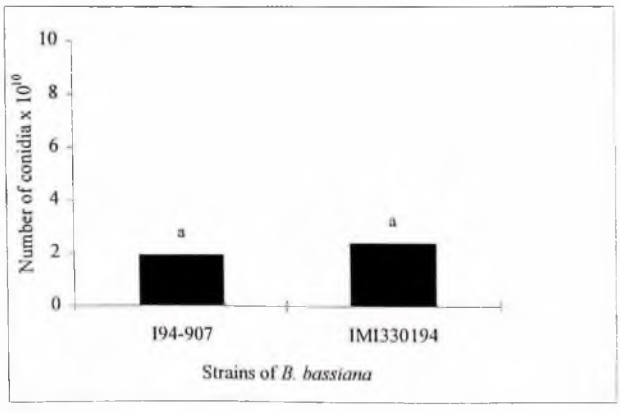

Figure 11. Mean number of conidia of *B. bassiana* strains per gram of conidial powder.

Means represented by bars headed by the same letters are not significantly different at the 5% level (Tukey's test in SAS).

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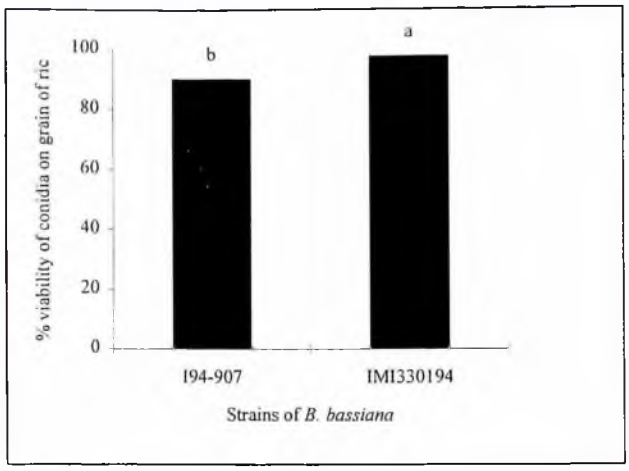

Figure 12. Mean percent viability of conidia of *B. bassiana* strains on rice grains.

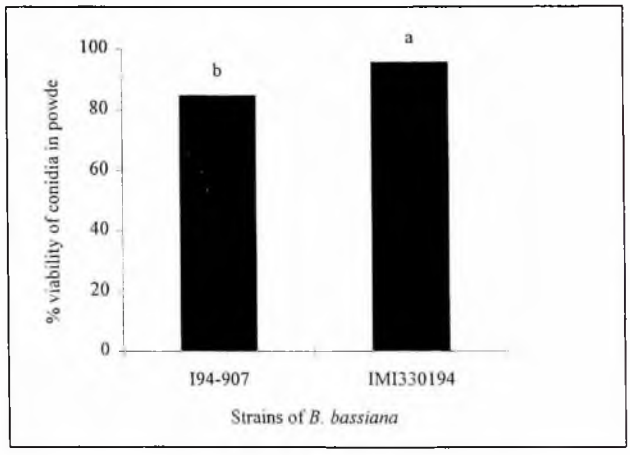

Figure 13. Mean percent viability of the conidia of *B. bassiana* strains in powder extracted from rice.

Means represented by bars headed by the same letters are not significantly different at the 5% level (Tukey's test in SAS).

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4.3.3. Comparative virulence of the strains I94-907 and IMI330194 of *B. bassiana* to *C. sordidus*

Mortality of adult weevils exposed to I94-907 inoculum started earlier and was significantly higher at day 7 after exposure compared to that of weevils exposed to IMI330194 inoculum and control at the 5% level (Figure 14a., Table 9., appendix 12a.). At days 14 and 21 this difference disappeared and the percentage mortality of adult weevils exposed to IMI330194 inoculum was higher than percentage cumulative mortality of weevils exposed to I94-907 inoculum and control (Figure 14a.). However the percentage cumulative mortality caused by the two fungal strains at days 14 and 21 after exposure was not significantly different at the 5% level (Table 9.). In contrast, the percentage cumulative mortality caused by the fungal strains was significantly higher compared with that of the control (Table 9.).

Adults exposed to the two strains showed clear growth of *B. bassiana* after death (Plate 8.) In contrast, dead adults in the control treatments did not show any signs of *B. bassiana* mycosis (Figure 14b.). Percentage cumulative adult weevils dead with signs of mycosis after exposure to the fungal inoculum (35.3-42.3%) were significantly higher than that exposed to the control (0.0%) (Table 10.; Appendix 13.). In contrast the percentage cumulative dead weevils with signs of mycosis after exposure to inocula of the two strains was not significantly different at the 5% level over the study period (Table 10.)

Mean percentage non-hatched eggs (54.9%) after exposure to I94-907 inoculum was higher compared with that exposed to control (44.3%) (Figure 15.). However this difference was not significant ($P > 0.05$; Appendix 14.). In contrast, mean percentage non-hatched eggs after exposure to IMI330194 inoculum (46.3%) was significantly higher than for those exposed to control (5.1%) (Figure 16. and Appendix 16). In fact, percentage non-hatched eggs after exposure to the fungal inocula was higher compared with that of the control. Also the level of percentage non-hatched eggs caused by the strain IMI330194 was higher compared with that caused by the strain

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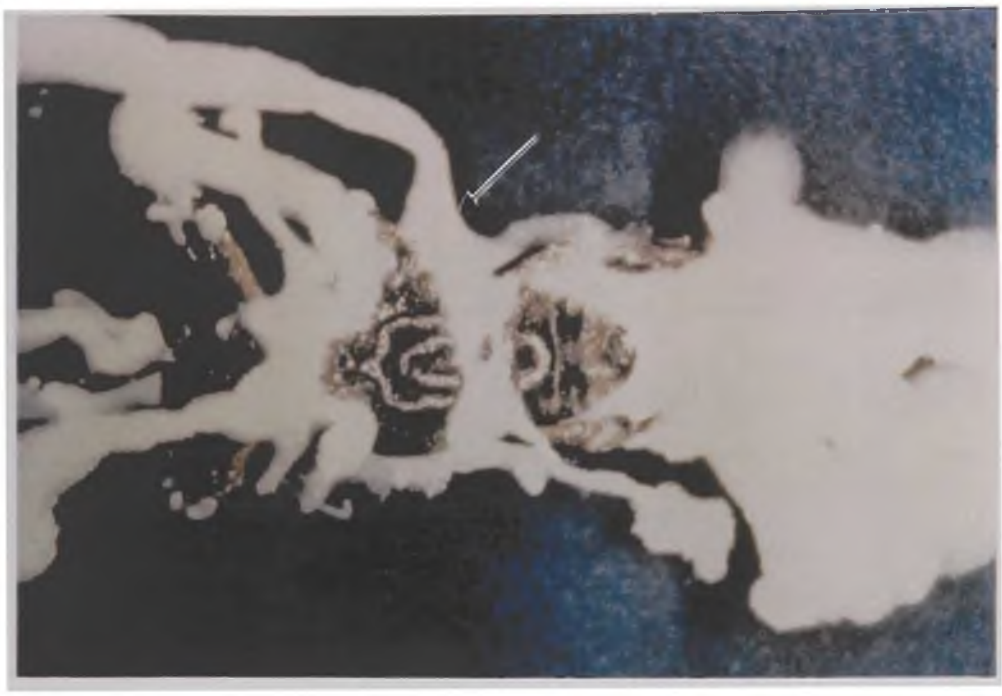

Plate 8. *C. sordidus* showing external growth of *B. bassiana* at death after exposure to *B. bassiana* inoculum.

Note: White mycelia of *B. bassiana* (arrow).

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Table 9. Mean percentage cumulative mortality of *C. sordidus* 7, 14 and 21 days after exposure to *B. bassiana* strains I94-907 and IMI330194.

Strain	Days after exposure		
	7	14	21
I94-907	12.7a	38.5a	50.9a
IMI330194	0.0b	52.2a	57.8a
control	0.0b	4.0b	6.4b

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS).

Table 10. Mean percentage cumulative dead adults with signs of mycosis 7, 14 and 21 days after exposure to *B. bassiana* strains I94-907 and IMI330194.

Strain	Days after exposure		
	7	14	21
I94-907	5.1a	29.7a	42.3a
IMI330194	0.0a	30.2a	35.3a
control	0.0a	0.0b	0.0b

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS).

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I94-907 with respect to percentage unhatched eggs of the control in each experiment.

Eggs exposed to the different fungal inoculum showed external growth of *B. bassiana* while those exposed to the control did not show any fungal growth. Mean percentage of eggs with signs of mycosis after exposure to the strains I94-907 and IMI330194 inocula (20.0% and 21.3% respectively) was significantly higher ($P < 0.01$) compared to that of eggs exposed to the control (0.0%) (Appendices 15. and 17.).

Mortality of larvae within the pieces of corm treated with I94-907 inoculum (45.5-54.6%) was significantly higher than mortality of larvae in pieces of corm treated with control (8.0-10.0%) (Figure 17.; Appendices 18. and 19.) at days 7 and 21 after treatment. In contrast mortality of larvae in pieces of corm treated with IMI330194 inoculum was higher (50.0-60.0%) than mortality of larvae in corm treated with control (25.0-30.0%) (Figure 18.). However, this difference was not significant ($P > 0.05$) (Appendices 20 and 21.). The level of larval mortality caused by the strain I94-907 in comparison with larval mortality in the control was higher than that caused by the strain IMI330194.

Dead larvae obtained from inoculum of I94-907 (36.4%) and IMI330194 (25.0%) showed clear growth of *B. bassiana* compared to 0.0% for the control.

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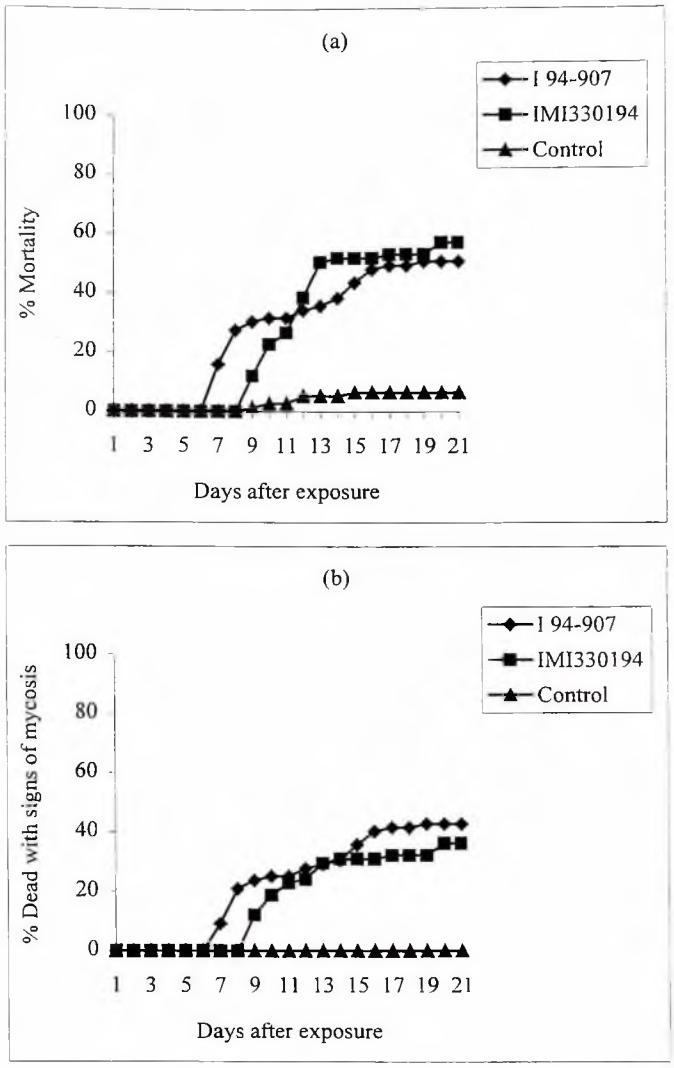

Figure 14. Percentage cumulative mortality of *C. sordidus adults* (a) and percentage dead adults of *C. sordidus* with signs of mycosis (b) after exposure to pseudostem traps treated with *B. bassiana* inoculum.

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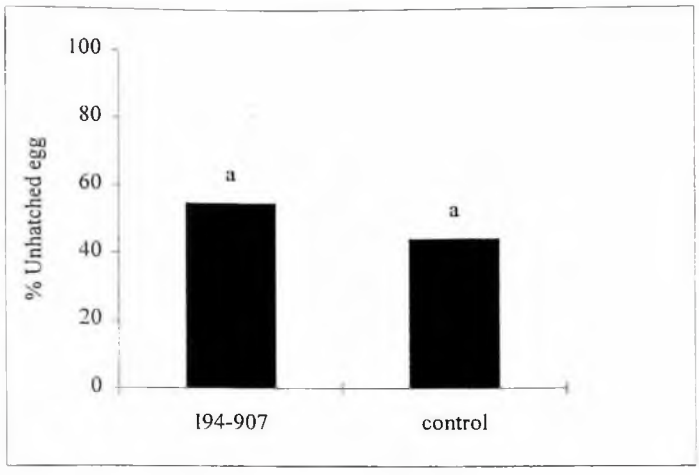

Figure 15. @Mean percentage non-hatched egg of *C. sordidus* 10 days after exposure to I94-907 inoculum.

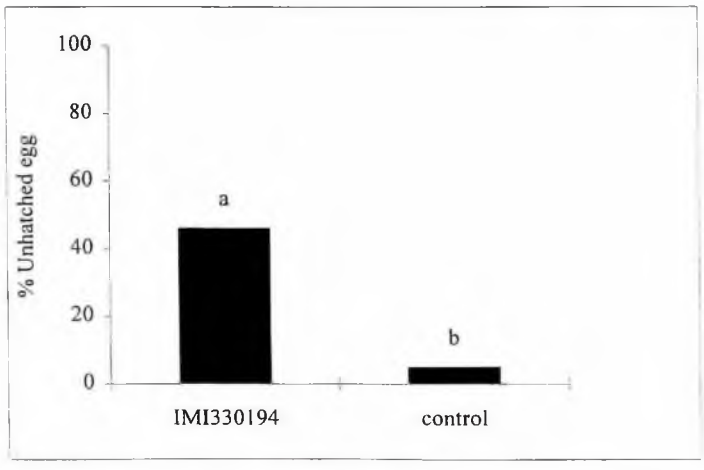

Figure 16. @Mean percentage non-hatched eggs of *C. sordidus* 10 days after exposure to IMI330194 inoculum.

Means represented by bars headed by the same letters are not significantly different at the 5% level (Tukey test, SAS). @angular means detransformed

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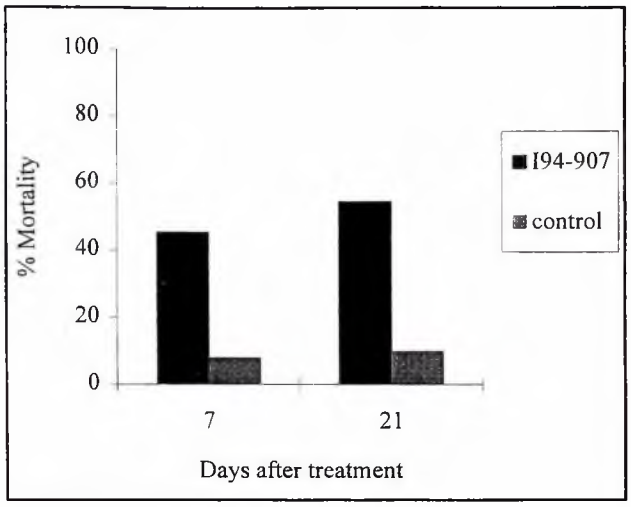

Figure 17. Mortality of larvae of *C. sordidus* within pieces of corm treated with *B. bassiana* strain I94-907 inoculum

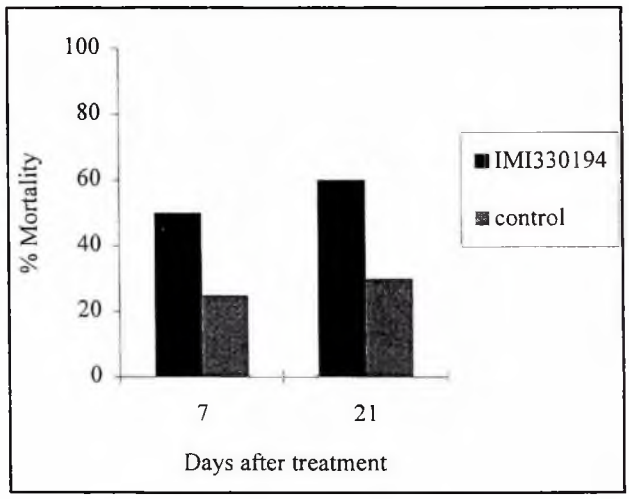

Figure 18. Mortality of larvae of *C. sordidus* within pieces of corm treated with *B. bassiana* strain IMI330194 inoculum.

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4.4. DISCUSSION

In the present study, out of the three strains tested, the strain ARSEF2624 caused the lowest mortality to *C. sordidus* adults even though it was isolated from the banana/plantain weevil. This result emphasises the variability in virulence of strain of *B. bassiana*. Pena *et al.* (1993) and Nankinga (1994) demonstrated similar variability for *B. bassiana* isolates on *C. sordidus*.

Delattre and Jean-Bart (1978) reported that *B. bassiana* strains from *C. sordidus* were more virulent to *C. sordidus* than those strains from other insects. In contrast, in this study, the strain ARSEF2624 isolated from *C. sordidus* was not virulent to the banana weevil while the strains I94-907 and IMI330194 isolated from *Cylas* spp. and *Hypothenemus hampei* Ferrari (Coleoptera: Scolytidae) respectively were virulent to the weevil. These two insect species are taxonomically related to *C. sordidus*, and also occur in plant tissues and soil, this might explain why *B. bassiana* strains isolated from these insects were virulent to the banana weevil.

In this study the strains I94-907 and IMI330194 were found to be pathogenic to all the stages of *C. sordidus*. Dead adults, larvae and eggs of *C. sordidus* showed signs of *B. bassiana* mycosis. Fungal growth at the weevil's surface would probably be an important source of inoculum to control the healthy weevil population in the plantain ecosystem. On the basis of the results obtained, the two strains I94-907 and IMI330194 proved to be potential candidates for controlling *C. sordidus*.

In the present study, the strains I94-907 and IMI330194 showed their potential to produce aerial conidia on rice grains. However, the strain IMI330194 gave the highest conidial yield with higher conidial viability compared to the strain I94-907. The conidial yield obtained in the present study (5×10^8 - 6×10^8 conidia/g rice) was lower than that reported in the literature. For example, a pilot factory production per year in Krasnodar was 22 tons Boverin (*B. bassiana* conidia plus an inert carrier, standardised at 6×10^9 conidia/g carrier) (Ferron, 1981). Antia-Londono *et al* (1992) reported a yield of *B. bassiana* conidia of 7.74×10^9 per gram of rice. Another example is that an

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average of 2.6 x 10^{13} of conidia of *B. bassiana* per kilogram of dry weight substrate was obtained using Mycotech system (Jenkins and Goettel, 1997b). The lower yield obtained in this study may be due to fungal strains and differences in the production techniques (i.e. substrate used during the production, water content of substrate). For example, Jenkins (1995) reported that moisture content of the rice substrate had a significant effect on the yield of conidia produced by *M. flavoviride*. Increasing moisture content resulted in increased yields up to 9.67 x 10^{8} conidia/g rice grains (200 ml of water kg^{-1} rice). Also the same author reported that light and substrate nutrient balance (i.e. carbon and nitrogen) had an effect on the conidial yield of *M. flavoviride*.

In this study, the strain selection was based on percentage mortality of adult weevils caused by the compared strains and their potential for mass production. However, the virulence of each strain on eggs and larvae of *C. sordidus* was also determined in a separate trial. In the present study, the strain IMI330194 of *B. bassiana* was found to be virulent on *C. sordidus* adults and had a higher level of productivity compared to the strain I94-907. Therefore, further investigation on the development of formulations of *B. bassiana* and their efficacy to control *C. sordidus* will be based on the strain IMI330194.

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CHAPTER 5

DEVELOPMENT AND EVALUATION OF DIFFERENT FORMULATIONS OF *B. bassiana* FOR THE CONTROL OF *C. sordidus* IN POT EXPERIMENTS

5.1. INTRODUCTION

Once a suitable fungal strain to control an insect pest has been identified from a bioassay procedure, the strain should then be tested on a small-scale in pots and then on a larger-scale in a field. Therefore, it becomes important to develop a fungal formulation that can be effective and persistent against the target insect under pot and field conditions. Formulation refers to the resultant composition when a pesticide is mixed with an active ingredient (s) (Tanada and Kaya, 1993). Any material that is added to a microbial control agent makes a formulated product (Tanada and Kaya, 1993). The ingredients added to the microbial control agent should contribute to the viability, stability, virulence and efficacy of the microbial-control agent and acceptance of the product by the user (Couch and Ignoffo, 1981).

For small-scale and large-scale trials, the amount of fungal formulation needed for replicated trials can be hundreds or thousands of times greater than that needed for laboratory trials. Therefore, the fungal spores must be mass produced. This implies, that a more efficient and economic technique for mass-production must be developed.

Facultative pathogens such as *Beauveria* spp. can easily be produced in vitro (Jenkins and Goettel, 1997b). Beauveria spp. survive as parasites or saprophytes in the absence of the hosts (Charles, 1941). This makes the fungus a suitable candidate for microbial insecticide development.

There are three basic methods for in vitro production of entomopathogenic fungi such as *Beauveria* spp.: liquid fermentation, production on solid substrates and, diphasic (liquid-solid phase) method that combine the advantages of the two other methods. In liquid fermentation or submerged culture, micro-organisms are grown in liquid

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medium either in shaken flasks or on a larger scale in deep tank fermenters. This system allows either the production of blastospores or dried mycelia or submerged conidia. Production on solid substrate enables the production of aerial conidia. The solid substrates can either be nutritive, such as rice, bran or wheat grains, or non-nutritive, such as vermiculite, sponge, or cloth (Jenkins and Goettel, 1997b). In diphasic production, fungal biomass is produced in liquid media and transferred onto solid substrate. This method is being used for the production of *M. flavoviride* in LUBILOSA programme at IITA (Jenkins and Goettel, 1997a).

The use of solid substrates for conidial production gives the opportunity to increase conidial yield by providing a larger surface area. However, Jenkins and Goettel (1997b) reported that the conidial yield is affected by a number of factors such as type of solid media, moisture content of solid media, the balance of nutrient elements in the media e.g. carbon/nitrogen (C/N) ratio which can easily affect the pH of the media (Jenkins, 1995) and aeration.

Considering the type of solid media, for example, it was reported that the yield of conidia on rice grains was higher than that on other substrates such as sweet potato, tapioca, papaya or coconut (Ibrahim and Low, 1993). It is also reported that adding groundnut oil to the rice grains improved the yield of conidia of *M. flavoviride* (Jenkins, 1995). The cost, simplicity and availability of media can be limiting factors for the production of fungi. For example liquid medium made of waste brewer's yeast plus ordinary sugar is preferable rather than liquid medium made of synthetic chemicals. Also using rice grain will be less costly than using agar. On this basis, cassava starch suspension and oil palm kernel cake (OPKC) were tested for the production of *M. flavoviride* for the control of grasshoppers in LUBILOSA programme. A promising result was obtained (Olga Idohou, Technician LUBILOSA Project, IITA, Cotonou, Personal communication, 1998).

Microbial agents can be formulated as wettable powder, liquids (aqueous or emulsifiable suspensions), dusts, granules and baits. The main factors to consider in choosing the type of formulation for effective pest control with fungi are the mechanisms of the infection and the habitat of the host (soil, terrestrial, or aquatic) (Soper and Ward, 1981). For example, liquid formulations (water or oil) are more

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preferable to the dusts for the control of aerial stages of insects such as aphids, grasshoppers and locusts. On the other hand, granular and liquid formulations (e.g. non-emulsifiable suspensions) were found to be the most appropriate formula for soil insects such as Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) (Soper and Ward, 1981). These formulations may be also effective against *C. sordidus*.

In the present Chapter, an investigation was made into the efficacy of different liquid formulations of *B. bassiana* prepared using conidial powder (CP) produced on rice grains and OPKC formulation of *B. bassiana*. In Chapter 4, the conidial yield obtained on rice grains during strain selection was lower compared to that generally cited in literature (Bartlett and Jaronski, 1988; Antia-Londono *et al.,* 1992). For this reason, a study was conducted to improve the conidial yield produced on rice grains during the solid-phase of mass production. The resulting CP was formulated together with water, groundnut oil plus kerosene, groundnut oil and clay soil formulations preparation for use against adult banana weevils. The liquid formulations investigated here were selected based on the background that water, mineral oil and groundnut oil plus kerosene formulations of *B. bassiana* might caused high mortality to *C. sordidus* adults (up to 98%) in the laboratory (Nankinga, 1994; Batista-Filho *et al.*, 1995; Troaré, 1995).

An alternative formulation strategy is to mix the conidia with the solid substrate used in the solid-phase of mass production. It was reported that, a rice grains formulation of *B. bassiana* applied to banana stumps in a field was 95% efficient against adult banana weevils (Gert Roland Fischer's Company COINBIOL-GRF, Guide for the use of *B. bassiana* to control Banana weevil in Brazil). Also, Nankinga (1999) reported >60% adult banana weevil mortality on banana suckers planted with maize grain formulation of *B. bassiana* in pot experiments. Rice and maize grain are edible and are relatively costly. The waste OPKC from the soap industry (Olga Idohou, Technician in LUBILOSA project, IITA, personal communication, 1997) was found to be a good substrate for *M. flavoviride* production. *B. bassiana* and *M. flavoviride* are taxonomically related and generally the same substrates were used for their production. Therefore, the present study investigated the production and formulation of *B. bassiana* on OPKC for the control of *C. sordidus*.

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The objectives in the present study were;

(i) To improve the yield of CP of *B. bassiana* strain IMI330194 on rice grains

(ii) To determine the potential for mass production of *B. bassiana* strain IMI330194 using alternative substrates

(iii) To develop the following formulations of *B. bassiana* strain IMI330194 against *C. sordidus* in pot experiments:

- Water-based formulation of conidial powder (W-CP)
- Groundnut oil plus kerosene-based formulation of CP (GOK-CP)
- Groundnut oil- based formulation of CP (GO-CP)
- Clay soil-based formulation of CP (CS-CP)
- OPKC-based formulation of conidia (OPKC-C)

And to evaluate the effect of the formulations against *C. sordidus* in pot experiments.

5.2. MATERIALS AND METHODS

5.2.1. Production of CP of *B. bassiana* strain IMI330194 on rice grains subjected to different treatments

The production of CP of *B. bassiana* strain IMI330194 was done using the liquid-solid phase technique described in Section 4.2.3.1. The following rice-grain treatments were compared with respect to production of *B. bassiana* conidia:

- Rice grains washed with cold running water, and the excess water drained off prior to autoclaving,
- Washed rice grains + water, heated until all the water was absorbed prior to autoclaving,
- Washed rice grains + water + oil (groundnut oil), heated until all the water was absorbed prior to autoclaving.

The different treatments were chosen according to those used in the LUBILOSA production system (Jenkins, 1995). The water and oil added to the rice grains was the standard (300 ml kg^{-1} of rice and 20 ml kg^{-1} of rice respectively) used by LUBILOSA for *Metarhizium* production (Jenkins, 1996).

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The production technique for the conidia in the different treatments (conidial suspension preparation for liquid medium inoculation, rice grain inoculation, incubation of inoculated rice and conidia sieving from rice grains) was the same as described in Section 4.2.3.1. although the drying period for sporulated rice grains was 14 days.

Data were taken on the weight of conidial powder kg^{-1} rice grain, the number of conidia g^{-1} of powder and the conidial viability using the procedure as described in Section 4.2.3.1. Data were transformed and analysed as described in Section 4.2.3.1.

5.2.2. Effect of different liquid and solid-phase media on the production of *B. bassiana* strain IMI330194

Two liquid-phase media, cassava starch and yeast plus sugar suspensions, were prepared and inoculated as follows:

- Cassava starch suspension: waste cassava starch suspension was collected from a local "gari" manufacturing industry. A quantity of 75 ml suspension was mixed either with or without 20 g of ordinary sugar and poured into 250 ml conical flasks. The flasks were then autoclaved at 120 °C and 120 kPa for 45 min. The autoclaved liquid medium was cooled under ambient conditions (24-25 °C) in the laboratory for approximately two hours. The cooled cassava starch suspension in each flask was inoculated with 1 ml of conidial suspension (approximately 10^7 conidia ml^{-1}) of the strain IMI330194 and incubated on a rotary shaker (Section 4.2.3.1).

- Yeast plus sugar suspension: brewers yeast + sugar suspension was prepared, distributed into conical flasks, autoclaved and inoculated with conidial suspension as described in Section 4.2.3.1.

Data were taken on the production of blatospores and hyphae in the liquid media three days after incubation (by observation of samples of liquid on a slide under the microscope x200)

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The incubated liquid media containing blastospores and hyphae were diluted with 75 ml of sterile tap water in each conical flask and were used to inoculate two solid media grounded OPKC and rice grains prepared as follows:

The OPKC was collected from a soap manufacturing industry at Abomey-Calavi near Cotonou, Benin. The OPKC was a waste product obtained after extraction of oil from oil palm kernel with a machine (Plate 9.). The OPKC was grounded into powder using an ordinary corn mill machine. A weight of 250 g of the powder was distributed into heat-resistant plastic bags bought from the local market. Tap water was added to the cake at different rates: 0, 25, 50, 75, 100% of the 250 g of OPKC in the plastic bags (v/w). The treated OPKC in plastic bags was autoclaved at 120 °C and 120 kPa for 45 min, then cooled at 24-25°C in the laboratory. After cooling, the substrates were inoculated with the diluted liquid medium containing blastospores and hyphae of *B. bassiana* strain IMI330194 (37.5 ml liquid medium per plastic bag). The plastic bags were folded loosely and put in plastic bowls (Jenkins, 1995) which were closed for 4 days and then opened for 14 days for air-drying (Plate 10.) in the laboratory at approximately 24°C.

Rice grains were found to be a good solid substrate for production of the strain IMI330194 conidia in the previous experiments so in the present trial, rice was used as a control solid substrate. Rice was bought from the local market and washed with tap water. A quantity of 250 g was placed into a plastic bag and autoclaved as for OPKC. The rice was inoculated with brewer yeast + sugar plus blastospores and hyphae suspension. The duration for incubation and air drying of the sporulated substrate was the same as for oil palm kernel cake.

The different treatments of OPKC and the control treatment (rice grains) are shown in Table 11. and were repeated three times.

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Table 11. Treatment combinations of liquid substrate, solid substrate and water amendments used for production of *B. bassiana* strain IMI330194

Suspension	Solid substrate (liquid phase)	Water added to the substrate (percentage of 250 g substrate) (v/w)
Yeast + sugar	OPKC	0
Yeast + sugar	OPKC	25
Yeast + sugar	OPKC	50
Yeast + sugar	OPKC	75
Yeast + sugar	OPKC	100
Cassava starch only	OPKC	50
Cassava starch only	OPKC	100
Cassava starch + sugar	OPKC	50
Cassava starch + sugar	OPKC	100
Yeast + sugar	rice	no water

Data were taken on:

- number of conidia per gram of solid substrate (see Section 4.2.3.1.)
- viability of conidia (see Section 4.2.3.1.)

The data were transformed and analysed as described in Section 4.2.3.1. for the different treatments comparison.

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Plate 9. Production of oil palm kernel cake resulting from kernel oil extraction.

Note:

Above: Oil palm kernel oil extraction machine with outlet of the cake that is collected in a bowl (arrow).

Below: Close up view of the oil palm kernel cake.

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Plate 10. *B. bassiana* strain IMI330194 growing on grounded oil palm kernel cake.

Note:

Left: Oil palm kernel cake without inoculation of *B. bassiana* (Control).

Right: Oil palm kernel cake inoculated with *B. bassiana* and incubated for 4 days and air dried for 14 days. The white colour shows mycelia and conidia of the fungus.

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5.2.3. Development of different formulations of *B. bassiana* strain IMI330194

Dry CP of the strain IMI330194 extracted from rice grains treated with water and heated until all the water was absorbed before autoclaving was used in subsequent liquid formulations. This method for conidial production was used because the results of experiment (Section 5.2.1.) showed that the yield of CP obtained was higher than that from other methods (Section 5.3.1.). The quantity of CP used for formulation was 2 g and contained 4 x 10^{10} conidia.

i) Water-based formulation of conidial powder (W-CP) of *B. bassiana* strain IMI330194

The W-CP formulation of *B. bassiana* strain IMI330194 was prepared by mixing 100 ml sterile distilled water (SDW) containing 0.05% Tween 80 with 2 g of the CP of the strain.

ii) Groundnut oil plus kerosene-based formulation of CP (GOK-CP) of *B. bassiana* strain IMI330194

The preparation of CP was as described above. Groundnut oil and kerosene bought in the local market were mixed with the CP. The mixture was prepared as follow: 50 ml of groundnut oil + 50 ml of kerosene + 2 g of CP of *B. bassiana* strain IMI330194.

iii) Groundnut oil-based formulation of CP (GO-CP) of *B. bassiana* strain IMI330194

The preparation of CP was done as previously described. Groundnut oil bought in the local market was mixed with the CP at a rate of 2 g of CP of *B. bassiana* strain IMI330194 plus 10 ml of groundnut oil to make the GO-CP formulation of the strain IMI330194.

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iv) Clay soil-based formulation of CP (CS-CP) of *B. bassiana* strain IMI330194

The CP was prepared as described earlier. Clay soil was collected from a gutter at the ARS, Kade. The suspension of clay soil was prepared by adding 100 g of clay soil to 50 ml SDW. For the preparation of CS-CP formulation of *B. bassiana* strain IMI330194, 20 ml of the suspension was mixed with 2 g of CP of strain IMI330194.

v) OPKC-based formulation of conidia (OPKC-C) of *B. bassiana* strain IMI330194

Unlike the above formulations where conidia produced on rice grains were extracted in form of CP and mixed with solvents, conidia produced on OPKC (Section 5.2.2.) were not extracted and the mixture conidia plus OPKC was used as formulation. The preparation of the OPKC-C of IMI330194 involves the method that used OPKC to which 100% water (v/w) was added and inoculated with yeast plus sugar suspension containing blastospore and hyphae (Section 5.2.2.). This method was used subsequently to obtain the OPKC-C of IMI330194 because the yeast + sugar suspension for hyphae and blastospore production was the standard used in the previous conidia production experiments and moreover the constitution of this suspension is well known. In addition the conidial yield in this treatment was comparable to that obtained from the treatments where cassava starch or cassava starch plus sugar was used (Table 14.).

5.2.4. Evaluation of the effect of different formulations of *B. bassiana* strain IMI330194 on adults of *C. sordidus* in pot experiments

5.2.4.1. Effect of W-CP and GOK-CP of *B. bassiana* strain IMI330194 on adults of *C. sordidus*

The W-CP and GOK-CP of *B. bassiana* strain IMI330194 used in the present study were developed as in Section 5.2.3.

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Sword suckers prepared as described in Section 3.2.2. were used as delivery material of the different formulations in plastic buckets (volume 25 l) filled with soil up to 2/3 of their volume.

The formulation application method depended on the formulation. Before planting, suckers were soaked for 1 min in the W-CP of IMI330194 (100 ml per sucker) and the remainder of the formulation was poured around the suckers in the plastic buckets. The GOK-CP of IMI330194 was applied using a spray bottle "Ajax vitre" (volume 650 ml) on the soil surface around the sucker in the buckets (100 ml per bucket). Blank groundnut oil plus kerosene (50:50 v/v), blank water (SDW plus 0.05% Tween 80) and CP of IMI330194 were used as checks for the formulations of IMI330194. The blank groundnut oil plus kerosene and blank water were applied as GOK-CP and W-CP respectively. The CP of IMI330194 (2g of CP per sucker) was poured into a locally bought polythene bag and the rhizome was introduced into the bag. The bag was held tight to the pseudostem and shaken until the CP covered the rhizome homogeneously. A control where nothing was applied to the suckers was also used.

Immediately after the formulation application, 20 adult banana weevils (10 males and 10 females) were released around the suckers in the soil. The soil in the plastic bucket was mulched with elephant grass to provide a conducive environment for the weevils (Plate 11b.) The plastic buckets were covered with mosquito mesh to prevent the insects from escaping (Plate 11a.). The treatments were replicated 4 times except, the GOK-CP treatment that was replicated 3 times because of insufficient formulation. Cut pseudostems, 15 cm in length and split lengthways into two, were used as traps. The traps were set and replaced for live weevil trapping at two day-intervals in the plastic buckets 26 days after weevil release. The live and dead weevils were collected from the buckets daily for 14 days. The live weevils were kept in Petri dishes (diameter 9 cm) containing pieces of corm, in the laboratory at 25-28°C. Dead weevils were removed from the Petri dishes daily. The suckers were uprooted 42 days after weevil release. Dead and live weevils were collected and counted. The dead weevils collected from the buckets and the Petri dishes were surface sterilised and incubated in Petri dishes as described in Section 4.2.2. to examine the external growth of *B. bassiana*. The percentage cumulative dead weevils (both dead weevils in the

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100 mm

Plate 11. Plastic buckets in which plantain suckers subjected to different treatments were planted and in which adult weevils were released.

Note:

(a) Plastic bucket covered with mosquito mesh to prevent the weevils from escaping.

(b) Elephant grass mulch around the suckers to provide a conducive environment for the weevils.

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bucket and laboratory) and percentage cumulative dead weevils with signs of *B. bassiana* mycosis 26 and 42 days after weevil release were calculated, angular tranformed and analysed as described in Section 4.2.2.

5.2.4.2. Effect of GO-CP and CS-CP of *B. bassiana* strain IMI330194 on adults of *C. sordidus*

The GO-CP and CS-CP of *B. bassiana* strain IMI330194 used were developed as in Section 5.2.3.

Plantain suckers prepared as described in Section 3.2.2., were coated with the different formulations. The coating of the suckers with the GO-CP and CS-CP of IMI330194 were done by smearing the formulations on the rhizome with a spoon. Blank groundnut oil, blank clay soil and CP of IMI330194 were used as checks for GO-CP and CS-CP of IMI330194. The blank groundnut oil and blank clay soil was applied as GO-CP and CS-CP of IMI330194. The CP of IMI330194 was applied as described in Section 5.2.4.1. A control where nothing was applied to the suckers was also used. The treated and non-treated suckers were then allocated to plastic buckets (volume 25 l) filled with soil up to 2/3 of their volume at random. Immediately after the suckers were planted, adult banana weevils were released in the plastic buckets as described in Section 5.2.4.1. The treatments were replicated 3 times. The buckets were placed in an open area outside.

The planted suckers were uprooted 18 days after weevil release. The number of dead weevils and the number of dead weevils showing signs of *B. bassiana* mycosis were recorded. The percentages dead weevils with and without signs of mycosis were calculated, angular transformed and analysed as described in Section 4.2.2.

5.2.4.3. Effect of OPKC-C of *B. bassiana* strain IMI330194 on adults of *C. sordidus*

The OPKC-C of *B. bassiana* strain IMI330194 was developed as previously described in Section 5.2.3. Plantain suckers free from *C. sordidus* as described in Section 3.2.2. were used to deliver the OPKC-C of IMI330194. The suckers were planted in plastic

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buckets (Section 5.2.4.1). At planting, the OPKC-C of IMI330194 was sprinkled around the suckers in the holes. A quantity of 40g of OPKC-C of IMI330194 containing 4 x 10^{10} conidia was used per sucker. CP of IMI330194 prepared as described in Sections 5.2.1. and 5.2.3. was used as check and was applied to the suckers as described in Section 5.2.4.1. A control (non-treated suckers) was also used. The treatment was replicated 4 times for the CP of IMI330194 and the non-treated suckers and 3 times for the OPKC-C of IMI330194 due to shortage of formulation. After planting the suckers in the buckets, 20 adult weevils (10 males and 10 females) were introduced into each plastic bucket. The suckers were uprooted 18 days after treatment and the number of dead and live weevils were counted. The percentage of dead weevils and sporulating dead weevils were recorded. The live weevils were collected and kept in Petri dishes containing pieces of corm in the laboratory at 25-28°C. Daily observations were performed on them until 35 days after treatment. Dead weevils were count, removed, surface sterilised and placed in new Petri dishes on moist tissue paper as described in Section 4.2.2. for external growth of *B. bassiana*. The percentage of overall mortality and dead weevils with sporulation including those of day 18 after treatment was calculated, angular transformed and analysed as described in Section 4.2.2.

5.2.4.4. Persistence in the soil of GO-CP and OPKC-C of *B. bassiana* strain IMI330194 against adults of *C. sordidus*

The GO-CP and OPKC-C of *B. bassiana* strain IMI330194 were infective to the adults of *C. sordidus* and in addition were easy to formulate and apply. Therefore, these formulations were selected and tested for their persistence in the soil against the adult weevils.

The formulations were prepared as in Section 5.2.3. and were as follows:
- 2 g of CP mixed with 10 ml of groundnut oil
- 40g of OPKC-C of IMI330194 (10^9 conidia/g)

Plantain suckers prepared as described in Section 3.2.2. were used as delivery materials of the formulations. The GO-CP of IMI330194 was used to coat the rhizome

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of the sucker by smearing the formulation on the rhizome with a spoon before planting while the OPKC-C of IMI330194 was sprinkled around the suckers in the holes. The CP of IMI330194 was used as check and was applied as described in Section 5.2.4.1. A control (non-treated sucker) was also used. The plantain suckers subjected to the different treatments were planted in plastic buckets (see Section 5.2.4.1.). All the treatments were replicated 4 times for each of the different days of persistence evaluation. The persistence of each formulation was monitored by releasing 20 insects (ten males and ten females) into each plastic bucket at day 0 (immediately after sucker treatment), day 14, day 28 and day 42 after sucker treatment. The top of the plastic buckets was covered with mosquito mesh to confine the insects around the suckers. Data were taken 35 days after each insect release on:

- number of dead insects,
- number of dead insects with signs of *B. bassiana* mycosis.

The percentages of dead insects with and without signs of mycosis were calculated, angular transformed and subjected to General linear models (GLM) procedure in SAS (SAS, 1997). Detranformed means were presented in Tables 19. and 20 and Figure 19.

The fungal persistence was measured on the basis of the percentage adult mortality and percentage dead *C. sordidus* with signs of mycosis.

5.3. RESULTS

5.3.1. Production of CP of *B. bassiana* strain IMI330194 on rice grains subjected to different treatments

Statistical analysis showed that the mean weight of conidial powder obtained on rice grains treated with water and groundnut oil was significantly lower at the 5% level than that obtained on untreated rice grain or rice grain treated with water (Appendix 22. and Table 12.). The mean weight of conidial powder (21.3g/kg rice) obtained with the rice grains treated with water only was higher compared with that from rice without water and, groundnut oil (18.9g/kg rice). However this difference was not significant at the 5% level (Table 12.). The mean number of conidia per gram of

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conidial powder obtained for the different treatments was not significantly different ($P > 0.05$) (Appendix 23.; Table 12.). The viability of conidia obtained in the different treatments was also not significantly different ($P > 0.05$) (Appendix 24.) and was ≥ 99% (Table 12.).

5.3.2. Production of hyphae and blastospores of the strain IMI330194 in yeast plus sugar, cassava starch plus sugar and cassava starch suspensions

The ability of the strain IMI330194 to produce mycelia and blatospores in brewers yeast and cassava starch suspensions is shown in Table 13. The different liquid media tested showed the same potential for blastospore production. On the other hand, the biomass of hyphae produced in the cassava starch plus ordinary sugar and water was reduced compared with that in the other suspensions.

5.3.3. Production of *B. bassiana* strain IMI330194 on OPKC

The mean number and mean viability of conidia from the different treatments (combination of liquid substrate, solid substrate and water amendments) were significantly different ($P < 0.01$) (Table 14. and Appendices 25. and 26.). The highest mean number of conidia per gram of solid substrate (1.8×10^9) was obtained with OPKC pre-treated with 100% water (solid substrate/water (w/v)) and inoculated with hyphae and blatospores produced in cassava starch plus sugar suspension. However, this mean was not significantly different at the 5% level from the mean number of conidia (1.5×10^9) obtained on rice grains inoculated with blastospore and hyphae produced in yeast plus sucrose suspension (control). The lowest number of conidia (1.0×10^7) was obtained on OPKC with no water added. The viability of conidia was positively affected by the amount of water added to the OPKC (Table 14.). The conidial viability was significantly lower on OPKC amended with less than 25% water (89%) compared to conidial viability (≥ 99%) on OPKC amended with ≥ 50% water.

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Table 12. Mean weight of conidial powder, number of conidia and conidia viability of *B. bassiana* strain IMI330194 produced on rice subjected to different treatments.

Treatment	weight of conidial powder (g) kg^{-1} rice	number of conidia g^{-1} powder	conidia viability (%)
Washed rice	18.9a	2.3×10^{10}a	99.7a
Washed rice grain + water	21.3a	2.4×10^{10}a	99.3a
Washed rice grain + water + oil	9.1b	2.2×10^{10}a	99.0a

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test)

Table 13. Production of blastospores and hyphae of *B. bassiana* isolate IMI330194 in brewers yeast and cassava starch suspensions.

Suspension	Blastospore	Hyphae
Yeast + sucrose + water	+ +	+ +
Cassava starch + sucrose + water	+ +	+
Cassava starch + water	+ +	+ +

\+ few
\+ + many

Table 14. Effect of production method on production and viability of conidia of *B. bassiana* strain IMI330194

Treatment			
Liquid + solid substrate solid substrate	Water added (%) v/w	Number of conidia per gram of rice grain	Viability of conidia (%)
Yeast + sugar + OPKC	0%	0.1×10^{8}d	89.30b
Yeast + sugar + OPKC	25%	5.7×10^{8}bc	89.62b
Yeast + sugar + OPKC	50%	9.7×10^{8}abc	98.34a
Yeast + sugar + OPKC	75%	1.6×10^{9}ab	96.50a
Yeast + sugar + OPKC	100%	1.6×10^{9}ab	98.94a
Cassava starch +OPKC	50%	8.2×10^{8}abc	96.37a
Cassava starch + OPKC	100%	1.7×10^{9}a	98.34a
Cassava starch + sugar + OPKC	50%	4.4×10^{8}c	95.71a
Cassava starch + sugar + OPKC	100%	1.8×10^{9}a	99.67a
Control (Yeast + sugar + rice grains + no water)		1.5×10^{9}ab	99.00a

Within a column means followed by the same letter are not significantly different at the 5% level (Tukey Test, SAS)

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5.3.4. Effect of W-CP and GOK-CP of *B. bassiana* strain IMI330194 on adults of *C. sordidus*

Statistical analysis showed that *B. bassiana* strain IMI330194 formulations had a significant effect on *C. sordidus* adults mortality (Appendices 27a. and 27b.). The percentage mortality of *C. sordidus* caused by GOK-CP of IMI330194 (73.0 and 84.7%) at day 26 and 42 after exposure was significantly higher than that caused by the W-CP of IMI330194 (25.9 and 31.2%) and the checks (<40%) (Table 15.). Percentage adult weevil mortality caused by the CP of IMI330194 (37.7%) was higher than that caused by W-CP of IMI330194 (31.2%) at day 42 after exposure. However, this difference was not significant at the 5% level (Table 15.). Mortality of adult weevils caused by the blank groundnut oil plus kerosene (38.2%) was higher than that due to W-CP of IMI330194 (31.2%) over the study period. However this difference was not significant at the 5% level (Table 15.). Mortality of *C. sordidus* due to the blank water (11.8%) and control where nothing was applied to sucker (1.1%) was lower compared with mortality due to the blank groundnut oil plus kerosene (38.2%)

Adult weevils exposed to the W-CP and GOK-CP and CP of IMI330194 showed clear growth of *B. bassiana* at death. Also adult weevils exposed to the blank groundnut oil plus kerosene showed signs of mycosis at death due to contamination during the formulation application or weevil release. The percentage dead weevils with signs of mycosis after exposure to the GOK-CP of IMI330194 (66.2%) was significantly higher than that of other treatments except for the CP (36.5%) where contamination had occurred (Table 16.; Appendices 27c. and 27d.). Dead weevils after exposure to the blank water and control where nothing was applied to suckers did not show any signs of mycosis.

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Table 15. Mean percentage cumulative mortality of *C. sordidus* at day 26 and 42 after exposure to W-CP and GOK-CP of *B. bassiana* strain IMI330194

Formulation	Days after exposure	
	26	42
IMI330194 GOK-CP	73.0a	84.7a
IMI330194 W-CP	25.9b	31.2b
IMI330194 CP (check)	37.7ab	37.7b
Groundnut oil + kerosene (check)	30.0b	38.2b
Water: SDW + 0.05% Tween 80 (check)	4.0c	11.8b
Control (no oil, water or conidia)	0.0c	1.1c

Means within a column followed by the same letter are not significantly different at the 5% level (Tukey's test, SAS).

Table 16. Mean percentage cumulative dead adults with signs of mycosis at day 26 and 42 after exposure to W-CP and GOK-CP of *B. bassiana* strain IMI330194

Formulation	Days after exposure	
	26	42
IMI330194 GOK-CP	59.7a	66.2a
IMI330194 W-CP	20.4b	20.4b
IMI330194 CP (check)	33.5ab	36.5ab
Groundnut oil + kerosene (check)	4.7bc	4.7bc
Water: SDW + 0.05% Tween 80 (check)	0.0c	0.0c
Control (no oil, water or conidia)	0.0c	0.0c

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS).

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5.3.5. Effect of GO-CP and CS-CP of *B. bassiana* strain IMI330194 on adults of *C. sordidus*

Analysis of variance (ANOVA) showed that there was a significant effect of formulation on adult weevils mortality ($P < 0.01$; Appendix 28a.). The mean percentage mortality of adult weevils exposed to CS-CP of IMI330194 was less than half that of adult weevils exposed to GO-CP and CP of IMI330194. However, this difference was not significant at the 5% level (Table 17.). The mean percentage mortality of *C. sordidus* exposed to the blank groundnut oil and blank clay soil and control where nothing was applied to sucker (< 3%) was significantly lower compared with that for GO-CP of IMI330194 (62.2%) and for CP of IMI330194 (61.4%).

Adult weevils exposed to GO-CP and CS-CP and CP of IMI330194 showed signs of *B. bassiana* mycosis at death while those exposed to blank groundnut oil, blank clay soil and control where nothing was applied to sucker did not show any signs of mycosis. The mean cumulative percentage of adult weevils with signs of mycosis at death after exposure to CP of IMI330194 (42.3%) was significantly higher than that of adult weevils exposed to other treatments except for GO-CP of IMI330194 (30.5%) (Table 17. and Appendix 28b.).

5.3.6. Effect of OPKC-C of *B. bassiana* strain IMI330194 on adults of *C. sordidus*

Analysis showed that there was a significant difference between mortality of *C. sordidus* caused by the OPKC-C of IMI330194, CP of IMI330194 and control where nothing was applied to sucker (Appendix 29a. and 29b.). The mortality of adult weevils caused by OPKC-C and CP of IMI330194 was significantly higher compared with that for control (nothing applied to sucker) over the study period (Table 18.). The mortality caused by OPKC-C of IMI330194 (40.5%) was lower than that due to CP of IMI330194 (54.9%) at day 18 after exposure but at day 35 after exposure the cumulative mortality caused by the OPKC-C of IMI330194 (93.1%) turned to be higher than that (86.6%) due to the CP of IMI330194 (Table 18.). However the mortality caused by the OPKC-C of IMI330194 and CP of IMI330194 was not significantly different at the 5% level over the study period. Mortality of *C. sordidus* caused by the OPKC-C of IMI330194 and CP of IMI330194 increased over time and

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was at the end of the study period (day 35 after weevil exposure to formulations) two times higher than that obtained at day 18 after weevil exposure.

Weevils dying from OPKC-C of IMI330194 and CP of IMI330194 showed signs of mycosis while those from the control where nothing was applied to sucker did not show any signs of mycosis. Dead weevils with signs of mycosis after exposure to OPKC-C of IMI330194 (20.2 and 52.5%) and CP of IMI330194 (36.6 and 57.9%) were significantly higher than that for control where nothing was applied to sucker (0.0%) (Table 18.; Appendices 29c. and 29d.).

5.3.7. Persistence in the soil of GO-CP and OPKC-C of *B. bassiana* strain IMI330194 against adults of *C. sordidus*

Analysis showed that there was a significant interaction between days of weevil release and formulation ($P < 0.01$) (Appendix 30a.). The effect of formulation on adult weevil mortality was significant ($P < 0.05$) with OPKC-C of IMI330194 giving higher mortality compared to the GO-CP of IMI330194 and checks from day 14 onwards (Figure 19. and Table 19.). For all treatments except OPKC-C of IMI330194, percentage mortality decreased with time. Mortality caused by the OPKC-C of IMI330194 increased to a maximum 83.1% on day 14 after weevil release and then decreased.

Dead adult weevils showed clear growth of *B. bassiana* after exposure to fungal formulations and CP of IMI330194. For the two fungal formulations and CP of IMI330194 treatments, the percent weevils with signs of mycosis decreased over time of persistence evaluation (Figure 19.). The percentage dead weevils with mycosis was significantly higher for days 14, 28 and 42 of persistence evaluation at the 5% level after exposure to OPKC-C of IMI330194 compared with that for the GO-CP of IMI330194 and CP of IMI330194 (Table 20 and Figure 19.; Appendix 30b.).

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Table 17. Mean percentage cumulative mortality and percentage cumulative dead *C. sordidus* with signs of mycosis at day 18 after exposure to GO-CP and CS-CP of *B. bassiana* strain IMI330194.

Formulation	Mortality (%)	Dead with mycosis (%)
IMI330194 GO-CP	62.2a	30.5ab
IMI330194 CS-CP	26.0ab	12.1b
IMI330194 CP	61.4a	42.3a
Groundnut oil	3.0b	0.0c
Clay soil	1.3b	0.0c
Control (no oil, clay soil and conidia)	1.1b	0.0c

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS).

Table 18. Mean percentage cumulative mortality and percentage cumulative dead adults of *C. sordidus* showing signs of mycosis 18 days and 35 days after exposure to OPKC-C of *B. bassiana* strain IMI330194.

Formulation	Mortality (%)		dead showing signs of mycosis (%)	
	18 days	35 days	18 days	35 days
IMI330194 OPKC-C	40.5a	93.1a	20.2a	52.5a
IMI330194 CP	54.9a	86.6a	36.6a	57.9a
Control (nothing)	0.6b	12.9b	0.0b	0.0b

Mean within a column followed by the same letters are not significantly different at the 5% level (Tukey's test).

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Table 19. Mean percentage cumulative mortality of *C. sordidus* after exposure to different formulations at day 0, 14, 28 and 42 after sucker treatment.

Formulation	Days of weevil release after formulation application			
	0	14	28	42
IMI330194 OPKC-C	61.3aB	83.1aA	61.0aB	22.0aC
IMI330194 GO-CP	69.9aA	16.0bB	5.9bBC	0.9bC
IMI330194 CP	62.0aA	24.7bB	12.3bBC	5.6bC
Control (nothing)	5.5bA	2.4cA	3.9bA	0.0bA

Within days of weevil release, means followed by the same small letters are not significantly different at the 0.05 level (pdiff statment in GLM procedure; SAS)
Within formulations, means followed by the same capital letters are not significantly different at the 0.05 level (pdiff statment in GLM procedure; SAS).

Table 20. Mean percentage dead adults of *C. sordidus* with signs of mycosis when exposed to different formulations at day 0, 14, 28 and 42 after sucker treatment.

Formulation	Days of weevil release after formulation application			
	0	14	28	42
IMI330194 OPKC-C	39.9aA	39.8aA	29.3aA	8.7aB
IMI330194 GO-CP	22.2bA	9.0bB	0.6bC	1.6bC
IMI330194 CP	37.7aA	11.9bB	3.1bBC	1.5bC
Control (nothing)	0.0cA	0.0cA	0.0bA	0.0bA

Within days of weevil release, means followed by the same small letters are not significantly different at the 0.05 level (pdiff statment in GLM procedure; SAS)
Within formulations, means followed by the same capital letters are not significantly different at the 0.05 level (pdiff statment in GLM procedure; SAS).

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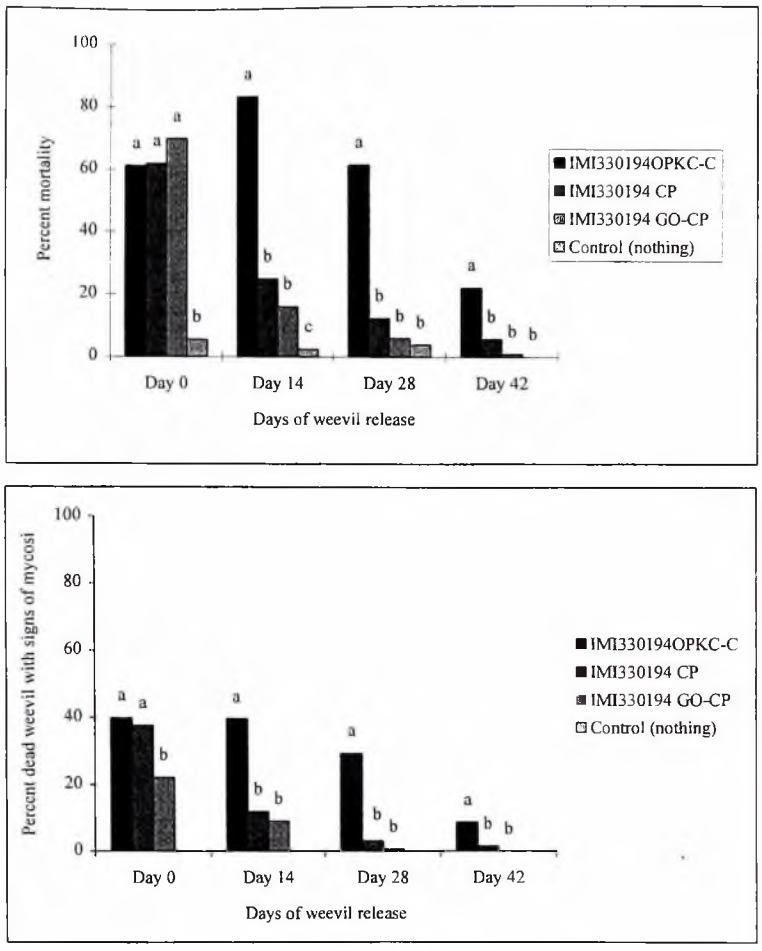

Figure 19. Percent mortality (top) and percent dead weevil with signs of mycosis (bottom) when exposed to different formulation at different days. No dead weevil was found with signs of mycosis when exposed to control (nothing). therefore no bar was shown for this treatment.

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5.4. DISCUSSION

In the present study, the yield of *B. bassiana* was significantly improved on rice grains treated with water. This yield was within the range of that reported by Bartlett and Jaronski (1988), and Antia-Londono *et al* (1992) but was low compared to that obtained in the Mycotech production system where the growing substrate was kept secret (Bradley *et al*, 1992). The findings were also in agreement with Jenkins (1995) who reported that increasing moisture content of rice grains by adding water increased conidial yield of *M. flavoviride*.

In contrast to results from mass production of *M. flavoviride* (Jenkins, 1995), groundnut oil had a negative effect on yield of *B. bassiana*. This indicates that the optimal condition (i.e. substrate nature) of mass production for fungi, even when taxonomically related, may not be the same. Optimizing production conditions through other variables such as aeration, or light or micro-nutrients could also have resulted in further increase in conidial yield as was shown for *M. flavoviride* production (Jenkins, 1995).

The study further showed that *B. bassiana* can be produced on waste products (cassava starch suspension as liquid medium and OPKC as solid substrate). Other researchers have had success with a range of solid substrates e.g. rice, maize, bean, wheat grains, tapioca (Anti-Londono *et al*, 1992; Ibrahim and Low, 1993; Batista-Filho *et al*., 1995) but these products are not economical if attempting to reduce production costs considerably. *B. bassiana* conidial yield obtained using cassava starch suspension and OPKC with 75-100% water (w/v) was comparable to that obtained on rice inoculated with yeast plus sugar suspension containing hyphae and blastospores and also other production systems (Bartlett and Jaronski, 1988; Antia-Londono *et al*., 1992). Therefore, OPKC could be used as a substitute for rice grains, resulting in substrate cost reduction. Also, cassava starch could be an alternative to brewers yeast for hyphal and blastospore production. *B. bassiana* can grow on a wide range of natural solid media therefore, further screening for media for both high productivity and availability may play an important role in production cost reduction.

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After the fungal pathogen has been selected, a formulation must be developed. The formulation must exhibit several characteristics to be successful. When applied to the target area, the pathogen should spread uniformly and quickly, and remain infective for a period of time (Soper and Ward, 1981). In this study, W-CP, GOK-CP, GO-CP, CS-CP and OPKC-C of IMI330194 were tested for their ability to kill adult weevils and in addition GO-CP and OPKC-C were tested for their persistence. All the fungal formulations and CP of IMI330194 (check) caused substantial mortality to the adult weevils (26-93%). In general, GOP-CP and GO-CP of IMI330194 were found to induce the highest weevil mortality immediately after application. This result is similar to that found by Batista-Filho *et al.* (1995). The authors observed the highest mortality of *C. sordidus* when exposed to *B. bassiana* oil formulation compared to other formulations. In addition, Prior *et al.* (1988) during their investigation on the efficacy of *B. bassiana* against the weevil *Pantorhytes plutus* Oberthür (Coleoptera: Curculionidae) in cocoa found an estimated value for LD_{50} for an oil formulation to be 36 times lower than that for the water formulation. In the present study, it is important to note that the percentage mortality caused by the blank groundnut oil and groundnut oil plus kerosene (3 and 38%) was more than double that of the blank water or control with nothing. This result was in agreement with that reported by Batista-Filho *et al.* (1995). He found that blank mineral oil can cause up to 33% mortality of *C. sordidus* against 0% for control (no formulation). In contrast, Traoré (1995) did not find any effect of groundnut oil plus kerosene on weevil mortality.

In this study, generally, adult weevils exposed to the fungal formulations showed clear growth of *B. bassiana*. In contrast, except in the first experiment of formulation evaluation, where contamination occurred in blank groundnut oil plus kerosene treatment, weevils exposed to the control with no conidia did not show any fungal growth. The percentage of insects showing growth of *B. bassiana* after exposure to GO-CP of IMI330194 was generally lower than that exposed to CP of IMI330194 treatments. It is possible that the conidia in CP were more easily picked by the weevils than when the conidia were mixed with a solvent or other ingredients. The external growth of entomopathogenic fungi such as *Beauveria* spp. is seen as a possible source of inoculum for the non-infested insect population and may also favour the spread of the disease (Nankinga, 1994; Traoré, 1995) that can lead to an epizootic situation.

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Throughout the different experiments conducted, the GO-CP, and OPKC-C of IMI330194 and CP of IMI330194 were found to be effective against adults of *C. sordidus* (Tables 17. and 18.) but the effect of the OPKC-C of IMI330194 against *C. sordidus* was highly persistent compared with the other formulations. The reason for this was that the solid substrate (OPKC) enhanced the fungal multiplication and viability. Traoré (1995) reported from a laboratory study that *B. bassiana* formulated in groundnut oil + kerosene could persist in the soil for 28 days with an infection rate of 51%. This result contrasts what was obtained in the present study with *B. bassiana* oil formulation. Possible reasons are that the experimental conditions and fungal strain, may affect the results. On the other hand, OPKC-C of IMI330194 persisted in the soil for 28 days with infection rate of 61%, which was higher than that reported by Traoré (1995). Since the banana weevil is a soil-borne insect and the adult weevil is cryptic, there is an advantage to using formulations that can enhance the fungal virulence in soil. Therefore, OPKC-C of IMI330194 having a potential to enhance conidia of *B. bassiana* multiplication in the soil with relatively long persistence could be a good formulation for the management of *C. sordidus* in the field.

In the following chapter investigations will be made on the potential of OPKC-C of IMI330194 for the management of adults of *C. sordidus* in a field.

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CHAPTER 6

FIELD EVALUATION OF SELECTED FORMULATION OF *B. bassiana* STRAIN FOR THE CONTROL OF *C. sordidus*

6.1. INTRODUCTION

Field testing is an essential step that needs to be undertaken before any experimental technique can be recommended for implementation (Shah, 1994). A field trial for a mycoinsecticide could be an arena trial (Bateman *et al.*, 1992) or a small-scale trial to demonstrate field infection or a medium to large-scale trial to show population control as well as field infection. Field experiments may be classified as "demonstrations" (one treated plot), "tests" (two replications), and proper "trials" (≥ 3 replications) (Shah, 1994).

Burges (1981) reported that many field experiments reveal the potential uses of the muscardine fungi (e.g. *Beauveria* spp. and *Metarhizium* spp.) provided that the technical difficulties limiting their mass-production are overcome. The efficacy of muscardine fungi is comparable to that of chemical insecticides with additional advantages of noticeable long-term insect pest limitation and little disturbance to the agroecosystem (Burges 1981). Entomopathogenic fungi such as *Beauveria* spp and *Metarhizium* spp. have the potential to grow, multiply and persist on the insect they kill. Also infected hosts can move from the infection point to another place, thus, spreading the pathogen throughout the pest's habitat. This may result in an increase of inoculum in the pest population possibly leading to an epizootic situation (Burges, 1981). In contrast to chemicals, re-application may not be necessary.

The movement of the infected weevils in the field is not known. The studies of Harris (1947) and Whalley (1957) on movement of healthy weevil, however, revealed that the banana/plantain weevil appears to be sedentary and its movement was confined within a radius 9 m of the original release point. Therefore there is a need to have

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information on the movement of weevils infected with *B. bassiana* and the spr the fungus in a field.

Studies in the previous chapters showed the high mortality of *C. sordidus* cau: the strain IMI330194 of *B. bassiana* in the laboratory and pot trials, and its p attributes for mass-production (high productivity, reported lower susceptibi contaminants). It was also found that OPKC-C and CP of IMI330194 were infective to *C. sordidus* adults than other formulations in pot experiments. For reasons, there is a need to carry out field trials to determine the effects of the OP and that of CP of IMI330194 in the field. Jaronski and Goettel (1997) reporte numerous environmental constraints including temperature, relative humidit ultraviolet radiation may affect the viability of fungal conidia and then limi efficacy of entomopathogenic fungi. The inoculum dose available for laborato pot experiments may not be adequate for field experiment, therefore, in this investigations were also made to determine the best dose of *B. bassiana* conic use in a field based on that used in the pot experiments.

From the foregoing, the objectives of current studies were to:

(i) Determine the dose of conidia needed for field studies using CP of *B. ba* strain IMI330194.

(ii) Determine the efficacy of OPKC-C of *B. bassiana* strain IMI330194 in where weevils were artificially released and a field naturally infeste weevils

(iii) Determine the spread of *B. bassiana* in a field using adult weevils artif infected with OPKC-C of *B. bassiana* strain IMI330194

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6.2. MATERIALS AND METHODS

6.2.1. Determination of the dose of conidia needed for field studies by comparing the effect of two doses of *B. bassiana* strain IMI330194 conidia on *C. sordidus* adults in a field where weevils were artificially released

A proper field trial was conducted at ARS, Kade between 19th June and 17th July 1997. Field preparation and planting distance were the same as described in Section 3.2.2. Three blocks were demarcated with three experimental plots each. Two doses, 2 g and 3 g of CP (2 x 10^{10} conidia g^{-1} powder) of *B. bassiana* strain IMI330194 (Sections 5.2.1 and 5.2.3.) were tested. Plantain suckers prepared as described in Section 3.2.2. were used as delivery material. The different treatments in the experiment were;

- Rhizome coated with 2 g of CP to give a dose of 4 x 10^{10} conida
- Rhizome coated with 3 g of CP to give a dose of 6 x 10^{10} conidia
- Rhizome non-coated with CP

The rhizomes were coated as described in Section 5.2.4.1. The treatments were randomised in each block. Each plot was planted with 12 suckers. Each sucker was infested with twenty adult banana weevils (10 males and 10 females) immediately after planting. Mulch from weeded *C. odorata* was provided around the suckers to retain the insects in place.

Observations were made daily from 5 to 28 days after weevil release, to determine the number of dead weevils at the soil surface around each sucker. The starting day of observation, 5 days after weevil release was chosen based on the laboratory results (Section 4.3.3.) and was the time when mortality of infected weevils usually started. The 28th day after weevil release was chosen to end the observations based on the biology study of *C. sordidus* (Section 3.3.1.). This was to avoid the overlapping of generations. The released weevil population was monitored in the experimental plots, 18-28 days after release by setting pseudostem traps at the base of each sucker to catch the weevils and evaluate their population level. The 18th day after weevil release was chosen based on pot experiment results (Sections 5.3.5.and 5.3.6.) and

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was a period when more than 50% of infected weevils died. The traps were inspected daily and the weevils caught in the traps were collected and kept in Petri dishes in the laboratory at 25-28°C. Daily observations were performed on the captured weevils in the laboratory until the end of the trial in the field.

Twenty-eight days after planting, the suckers were uprooted. The dead and live weevils in the planting hole were collected and counted. Dead weevils collected at the soil surface, from Petri dishes in the laboratory and from the planting holes, were surface sterilised and incubated in Petri dishes as described in Section 4.2.2. to determine the external growth of *B. bassiana*. Percentages were calculated for the following: cumulative mortality of *C. sordidus* at soil surface; dead weevils with signs of mycosis at the soil surface; cumulative mortality of *C. sordidus* in the laboratory; dead weevils with signs of mycosis in the laboratory; cumulative mortality of *C. sordidus* in planting holes and dead weevils with signs of mycosis in the planting holes. The overall percentage mortality (soil surface, laboratory and planting holes) and overall percentage dead weevils with signs of mycosis over the experimental period of 28 days were calculated. The numbers of eggs and larvae in the uprooted suckers were counted and recorded.

The percentages were angular transformed and analysed using ANOVA procedure in SAS. The numbers of eggs and larvae were subjected to GENMOD procedure (log linear models) for comparison (SAS, 1997). Detransformed means were presented in Section 6.3.1.

6.2.2. Effect of OPKC-C of *B. bassiana* strain IMI330194 on adults of *C. sordidus* in a field where weevils were artificially released

The study was carried out at ARS, Kade between June and July 1998. Field preparation and planting distance were the same as described in Section 3.2.2. The plantain suckers used were prepared as described in Section 3.2.2. OPKC-C of IMI330194 and CP prepared as described in Sections 5.2.1.and 5.2.3. were used.

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Treatments in the experiment were:

- Plantain sucker planted in hole containing 60g of OPKC-C of IMI330194 (10^9 conidia g $OPKC^{-1}$).
- Rhizome coated with 3g of CP (6×10^{10} conidia) to give the same dose of conidia as for the OPKC-C of IMI330194. This treatment was used as check for the OPKC-C of IMI330194.
- Untreated plantain suckers.

Three blocks were demarcated with three experimental plots each. The treatments were randomised in each block. Each plot was planted with 12 suckers. Each sucker was infested with adult weevils as described in Section 6.2.1. Observations, data collection and analysis followed the same methodology as described in Section 6.2.1.

6.2.3. Effect of OPKC-C of *B. bassiana* strain IMI330194 on adults of *C. sordidus* in a field naturally infested with weevils

A field trial was conducted from 22nd June to 21st August 1998 to determine the efficacy of OPKC-C of *B. bassiana* strain IMI330194 in a plantain field naturally infested with *C. sordidus*

A field survey was carried out at Akanteng in Eastern region of Ghana in order to determine the presence of *C. sordidus* prior to tests on the effect of *B. bassiana* on *C. sordidus* in a field naturally infested with weevils. The presence of *C. sordidus* in plantain fields was determined by using pseudostem traps of 15 cm long, split in half (lengthwise) (Ogenga-Latigo and Bakyalire, 1993). The pseudostem traps were placed at the bases of plantain plants. The traps were inspected and replaced at 3 day intervals. The number of *C. sordidus* per trap was counted and samples of them were taken to determine the natural incidence of *B. bassiana*. One field with 2 year old plantain stands was selected due to the high incidence of weevil (2 weevils per trap per three days). The field was in the forest zone and was formerly cropped with cocoa which was burnt in 1994 and was re-planted with the local plantain cultivar, Apantu-pa (False- horn) in 1996. Cocoa, Cassava and cocoyam were planted between the

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plantain plants in 1997. The main weed in the field was *C. odorata*. Prior to trial establishment and one month later the whole field was weeded.

Within the selected plantain field, nine plots of 30 x 30 m each were demarcated. Twelve plantain suckers prepared as described in Section 3.2.2. were treated as follows and planted between the old plantain plants in each plot:

-Suckers planted in holes containing OPKC-C of IMI330194,

-Rhizome of suckers coated with CP of IMI330194 prior to planting,

-Non-dusted suckers and no conidia in planting hole.

The different treatments were assigned to the experimental plots randomly, with three replicate plots per treatment. Traps were set at the bases of the planted suckers, 7 days after planting. They were inspected and replaced at 2 day intervals to collect live adult weevils. The collected live weevils were kept in Petri dishes with pieces of corm in the laboratory at 25-28°C. The dead weevils in the Petri dishes were counted and removed daily and incubated on moist tissue paper in Petri dishes to determine the external growth of *B. bassiana*.

Sixty days after planting, the plants were uprooted and data were taken on:

- The number of plants attacked by the weevils
- The number of plants killed by weevil attack
- The number of larvae of *C. sordidus* in the plants.

The proportion of dead weevils and proportion of dead weevils with signs of mycosis were analysed using Generalised Linear Models (GENMOD logit model) in SAS. The number of larvae counted in the plants were analysed using GENMOD log linear model procedure in SAS that gives the log-likelihood χ^2. The percentages of plants attacked by the weevil and of plants killed by weevil attack were calculated.

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6.2.4. Evaluation of the spread of *B. bassiana* in a plantain field using adult weevils artificially infected with OPKC-C of *B. bassiana* strain IMI330194

The study was carried out from 23rd June to 25th August 1998 at Akanteng in a 2 year-old plantain field of about 2 ha. Eight hundred adult banana weevils were collected from this plantain field using pseudostem traps. The weevils were divided into two batches of 400 (200 males and 200 females) each. Weevils from one batch were infected with OPKC-C of *B. bassiana* strain IMI330194 (Section 5.2.3.). The infection process took place in a plastic bucket (Section 5.2.4.1.), each containing a pared sucker of Apentu-pa in soil with 60g of OPKC-C of IMI330194 in planting hole. After planting, the weevils from one batch were released around the sucker base and the bucket was covered as in Section 5.2.4.1. The weevils from the second batch were released in plastic buckets containing a plantain sucker planted in soil without OPKC-C of IMI330194. The infected weevils were marked on their left elytra and the non-infected were marked on their right elytra, by scratching two lines.

The weevils were collected from the buckets 4 days after treatment and released in the centre of the plantain field. One day after weevil release, pseudostem traps were set at the bases of eight plantain plants (one trap per plant) at regular fixed distances (3, 6, 9, 12,..... 69 m) from the field centre.

The traps were inspected daily for 3 days and the number of marked weevils alive or dead in the traps was counted and the live weevils were left where they were found. The dead weevils were collected with forceps and placed in Petri dishes and carried to the laboratory to check for the external growth of *B. bassiana*. The traps were replaced at 3-day intervals. This frequency of trap setting and inspection was continued for two months. The marked weevils were also sought for in rotten plantain materials throughout the field and their numbers recorded. The counts of the marked weevils at the fixed sampling distances and over the study period were subjected to regression analysis in GENSTAT (GENSTAT, 1993).

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6.3. RESULTS

6.3.1. Determination of the dose of conidia needed for field studies by comparing the effect of two doses of *B. bassiana* strain IMI330194 conidia on *C. sordidus* adults in a field where weevils were artificially released

The overall percentage cumulative mortality of *C. sordidus* (> 50%) after exposure to the conidial doses was significantly higher than that of *C. sordidus* exposed to no conidia (< 4%) (Table 21. and Appendix 31a.). The mortality of *C. sordidus* exposed to the conidial dose 6 x 10^{10} (68.2%) was higher than that (53.4%) of weevils exposed to 4 x 10^{10} conidia. However this difference was not significant at the 5% level (Table 21.). Percentage cumulative mortality of *C. sordidus* in planting holes followed a similar pattern to overall percentage cumulative mortality (Table 22. and Appendix 32a.). The percentage cumulative mortality on the soil surface was higher for adult weevils exposed to the conidial doses (> 20%) compared to that of adult weevils exposed to no conidia (< 1%) (Figure 20.). The cumulative mortality of *C. sordidus* exposed to the conidial dose 6 x 10^{10} at the soil surface was higher compared with that of 4 x 10^{10} conidia over the study period (Figure 20.)

The overall percentage of adult weevils with signs of mycosis after death (59.9%) was significantly higher ($P < 0.01$) for weevils exposed to the dose 6 x 10^{10} conidia compared with that (31.9%) of weevils exposed to the dose 4 x 10^{10} conidia or no conidia (Table 21. and Appendix 31b.). The percentage adult weevils with signs of mycosis in planting holes followed a similar pattern to overall percentage adult weevils with signs of mycosis (Table 22. and Appendix 32b.).

The cumulative percentage live weevils collected (approximately 40%) after exposure to the conidial doses was half of that for weevils exposed to no conidia (Figure 21.).

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Table 21. Mean percentage mortality and dead adults of *C. sordidus* with signs of *B. bassiana* mycosis, 28 days after exposure to two doses of *B. bassiana* conidia in a field.

Dose of conidia	Mortality (%)	Dead adults with signs of mycosis (%)
6×10^{10}	68.2a	59.9a
4×10^{10}	53.4a	31.9b
0	3.1b	0.0c

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS).

Table 22. Mean percentage cumulative weevil mortality and percentage dead adults of *C. sordidus* with signs of mycosis in planting holes, 28 days after exposure to two doses of *B. bassiana* conidia in a field.

Dose of conidia	Mortality (%)	Dead with signs of mycosis (%)
6×10^{10}	28.9a	26.0a
4×10^{10}	21.8a	13.1b
0	0.2b	0.0c

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS).

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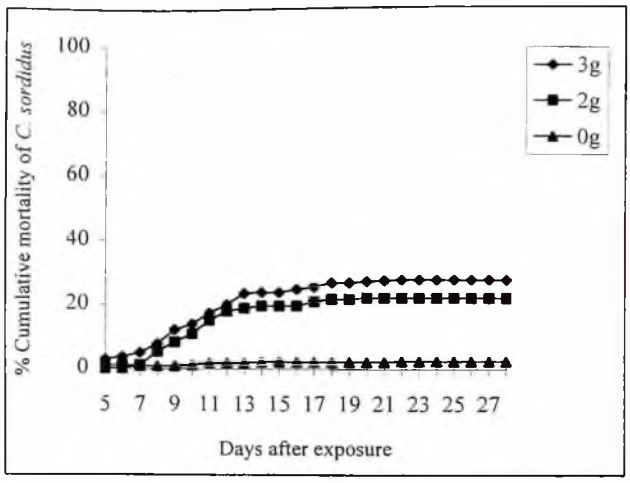

Figure 20. Percentage cumulative mortality of *C. sordidus* at the soil surface 5-28 days after exposure to two doses of conidia of the strain IMI330194 in a field.

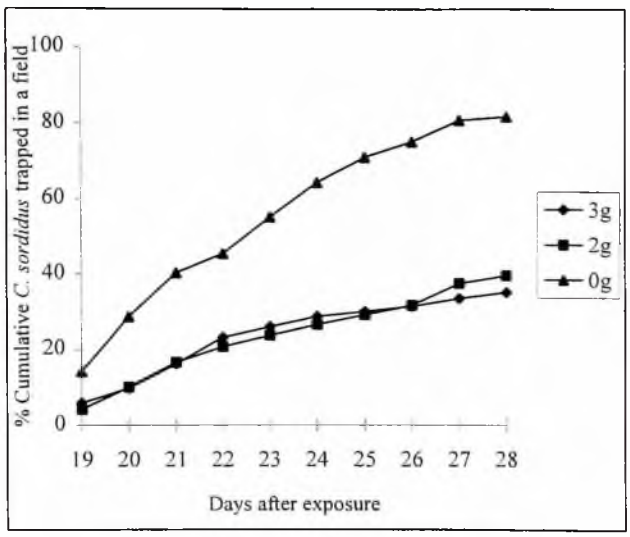

Figure 21. Percentage adults of *C. sordidus* recaptured with pseudostem traps 19-28 days after exposure to two doses of conidia of the strain IMI330194 in a field.

Note:

3g = 6×10^{10} conidia/rhizome

2g = 4×10^{10} conidia/rhizome

0g = no conidial powder

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The number of eggs collected from the plantain suckers treated with the different doses of conidia was not significantly different (χ^2 = 4.50; 2df; P>0.05). The number of larvae found in the plantain suckers treated with the different doses of conidia was significantly lower compared to that obtained in suckers not treated with conidia (χ^2 = 7.19; 1df; P < 0.01).

6.3.2. Effect of OPKC-C of *B. bassiana* strain IMI330194 on adults of *C. sordidus* in a field where weevils were artificially released

The overall percentage mortality of *C sordidus* (75.5%) after exposure to suckers coated with CP or suckers treated with OPKC-C of IMI330194 was significantly higher than that of *C. sordidus* exposed to the control (1.0%). There was no significant difference between mortality caused by the OPKC-C and the CP of IMI330194 (Appendix 33a. and Table 23.).

The cumulative percentage mortality of adult weevils caused by OPKC-C and CP of IMI330194 (28.3 and 27.2% respectively) in planting holes was significantly higher compared with that (0.1%) in the control (no conidia) (Appendix 34a and Table 24.). There was no significant difference between mortality caused by OPKC-C and CP of IMI330194 in planting holes at the 5% level (Table 24.). Cumulative percentage mortality on the soil surface was greater for adult weevils exposed to suckers coated with CP of IMI330194 compared with that of weevils exposed to OPKC-C of IMI330194 or the control where no conidia was applied to sucker (Figure 22.). Also an increase in cumulative adult mortality was observed after treatment with OPKC-C of IMI330194 which lagged behind that observed for CP of IMI330194 in time. The percentage live adults trapped was greater for the OPKC-C of IMI330194 than for the CP of IMI330194 (Figure 23.).

The overall cumulative percentage dead weevil with signs of mycosis for the OPKC-C of IMI330194 (40.5%) and for CP of IMI330194 (39.5%) was not significantly different at the 5% level (Table 23.). On the other hand, the overall cumulative percentage dead weevils with signs of mycosis caused by the control with no conidia

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(0.0%) was significantly lower compared with that caused by OPKC-C and CP of IMI330194 (Table 23. and Appendix 33b). The Percentage dead weevils with signs of mycosis in planting holes followed a similar pattern to the overall percentage dead adult weevils with signs of mycosis (Table 24. and Appendix 34b).

The number of larvae of *C. sordidus* collected from the sucker treated with the OPKC-C, CP of IMI330194, and control (no conidia) was significantly different ($\chi^2 = 24.17$; 2df; $P < 0.01$). The lower number was obtained from the suckers treated with OPKC-C of IMI330194.

Eggs of *C. sordidus* were not found on treated and non-treated suckers in this study. Therefore there was no analysis on number of eggs.

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Table 23. Mean percentage cumulative weevil mortality and percentage dead *C. sordidus* with signs of mycosis, 28 days after exposure to OPKC-C of *B. bassiana* strain IMI330194 in a field.

Formulation	Mortality (%)	Dead with signs of mycosis (%)
IMI330194 CP	75.5a	39.5a
IMI330194 OPKC-C	75.5a	40.5a
Control (no conidia)	1.0b	0.0b

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS)

Table 24. Mean percentage cumulative weevil mortality and percentage dead of *C. sordidus* with signs of mycosis in planting holes, 28 days after exposure to OPKC-C of *B. bassiana* strain IMI330194 in a field.

Formulation	Mortality (%)	Dead with signs of mycosis (%)
IMI330194 CP	27.2a	11.4a
IMI330194 OPKC-C	28.3a	10.7a
Control (no conidia)	0.1b	0.0b

Means within a column followed by the same letters are not significantly different at the 5% level (Tukey's test, SAS)

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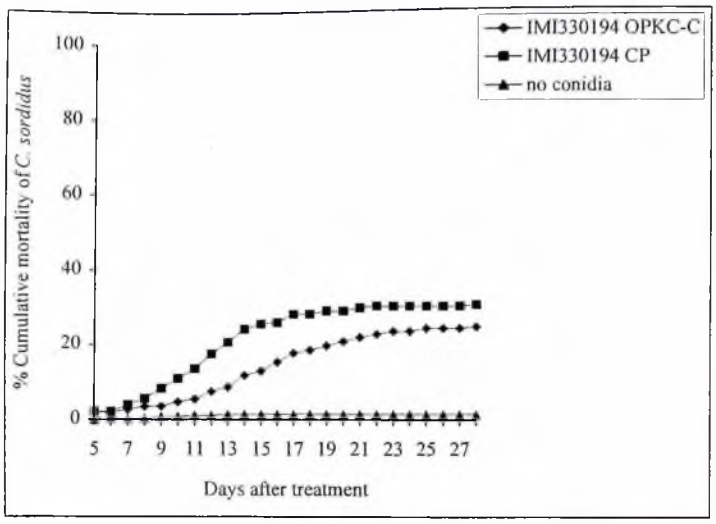

Figure 22. Percentage cumulative mortality of *C. sordidus* at the soil surface 5-28 days after exposure to OPKC-C of *B. bassiana* strain IMI330194 in a field.

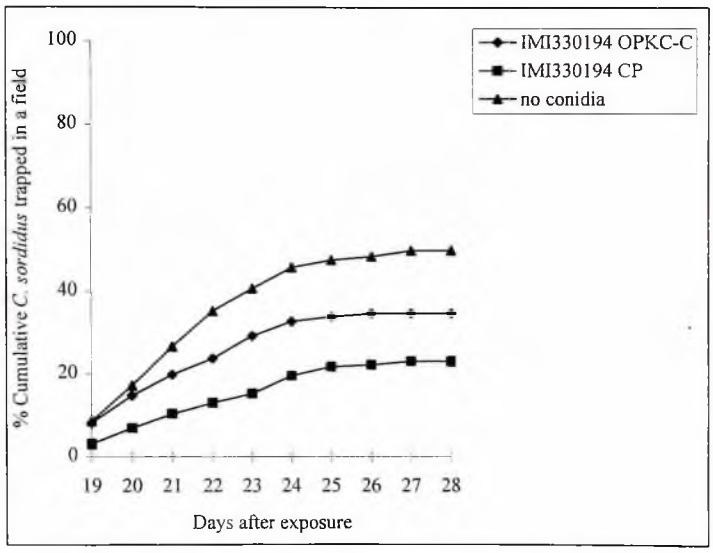

Figure 23. Percentage adult of *C. sordidus* recaptured with pseudostem traps 19-28 days after exposure to OPKC-C of *B. bassiana* strain IMI330194 in a field.

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6.3.3. Effect of OPKC-C of *B. bassiana* strain IMI330194 on adults of *C. sordidus* in a field naturally infested with weevils

Table 25. shows percentage cumulative mortality and percentage cumulative mycosis of adult weevils in the laboratory after collection from plantain suckers treated with OPKC-C of IMI330194 or plantain suckers treated with CP of IMI330194 or suckers treated with no conidia. The percentage mortality of adult weevils trapped live from suckers treated with the OPKC-C and CP of IMI330194 and control suckers treated with no conidia were significantly different ($\chi^2 = 6.18$; 2df; $P < 0.01$). The contrasts between the different treatments for the mortality of *C. sordidus* are shown in Table 25. The mortality of *C. sordidus* was significantly higher when collected from plantain suckers planted with OPKC formulation of IMI330194 (41.7%) than when collected from suckers treated with the CP of IMI330194 (5.7%) and suckers not treated with conidia (3.3%) (Tables 25. and 26.).

During the study period the growth of *B. bassiana* on the OPKC around the plantain suckers (Plate 12.) was observed while no growth of the fungus was observed for the CP and control (no conidia) treatments. The total number of *C. sordidus* larvae collected from treated and non-treated suckers is shown in Table 27. The number of larvae collected from the suckers treated with OPKC-C of IMI330194 (6) was significantly lower than that collected from suckers treated with the CP of IMI330194 (26) and non-treated suckers (31) ($\chi^2 = 6.56$; 2df; $P < 0.05$; Table 28.).

Table 29. shows the percentage of plantain suckers attacked by the weevils and the percentage that had died from the weevil attack in the different treatments, 60 days after planting. None of the suckers treated with the OPKC-C of IMI330194 died while 16.7% and 19.4% of treated suckers with CP of IMI330194 and non-treated suckers died respectively (Table 29.).

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Table 25. Percentage mortality and percentage dead adults of *C. sordidus* with signs of mycosis in the laboratory after collection from non-treated and treated suckers with OPKC-C of *B. bassiana* strain IMI330194 in a field.

Formulation	Mortality (%)	dead adults with signs of mycosis (%)
IMI330194 OPKC-C	41.7	33.3
IMI330194 CP	5.7	4.3
no conidia	3.3	0.0

Table 26. Contrasts between OPKC-C of *B. bassiana* strain IMI330194 and control treatments, for mortality of *C. sordidus* collected from the experimental plots.

Contrast	log-likelihood df	χ^2	Pr>Chi
No conidia vs Conidia	1	3.1530	0.0758
No conidia vs IMI330194 OPKC-C	1	5.6328	0.0176
No conidia vs IMI330194 CP	1	0.5518	0.4576
IMI330194 OPKC-C vs IMI330194 CP	1	4.2200	0.0400

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Table 27. Total larvae of *C. sordidus* collected from plantain suckers treated with or without OPKC-C of *B. bassiana* strain IMI330194, 60 days after planting.

Formulation	Number of larvae
IMI330194 OPKC-C	6
IMI330194 CP	26
No conidia	31

Table 28. Contrasts between OPKC-C of *B. bassiana* strain IMI330194 and controls for number of *C. sordidus* larvae collected from plantain suckers, 60 days after planting.

Contrast	df	log-likelihood χ^2	Pr>Chi
No conidia vs Formulation	1	2.2686	0.1320
No conidia vs OPKC + IMI330194	1	5.3416	0.0208
IMI330194 OPKC-C vs IMI330194 CP	1	4.8127	0.0283
No conidia vs IMI330194 CP	1	0.0141	0.9055

Table 29. Percentage of plantain suckers attacked and those killed by larvae of *C. sordidus* 60 days after been treated with OPKC-C of *B. bassiana* strain IMI330194 in field naturally infested with weevils.

Formulation	Percentage of suckers attacked	Percentage of suckers dying
IMI330194 OPKC-C	5.6	0.0
IMI330194 CP	25.0	16.7
No conidia	33.3	19.4

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Plate 12. White growth of *B. bassiana* strain IMI330194 on OPKC around a plantain sucker after application of OPKC-C of IMI330194.

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6.3.4 Evaluation of the spread of *B. bassiana* in a plantain field using adult weevils artificially infected with OPKC-C of *B. bassiana* strain IMI330194

Figure 24. shows the regression lines for the count of laboratory-infected or non-infected *C. sordidus* adults in a plantain field by sampling distance from 2 to 63 days after release. The slopes of the regression lines for the count of the infected and non-infected weevil at the sampling distances were significantly different ($t_{738} = 5.74$; $P < 0.001$, Appendix 35.). As the distance increased, the number of insects recovered decreased for both infected and non-infected weevils. The number of infected weevils at any sampling distance was consistently lower than that of non-infected (Figure 24.). Similarly, Figure 25. shows regression lines for daily counts of the released infected and non-infected weevils in the plantain field over 63 days. The daily count of the non-infected weevils remained constant while that of the infected weevils decreased over time. The number of non-infected weevils recovered daily was higher than that for the infected weevils (Figure 25.). The slope of the regression line for the daily counts of infected weevils was significantly different from that of the uninfected weevils ($t_{738} = 4.86$; $P < 0.001$, see Appendix 35.). No *C. sordidus* released were found further than 18 m from the release point. Figure 26. shows the mortality and sporulation of the infected and non-infected weevils trapped from different sampling distances, 63 days after release in the plantain field. The number of dead weevils and those that showed signs of mycosis obtained at 3m from the release point was considerably higher than the number at 6-18m.

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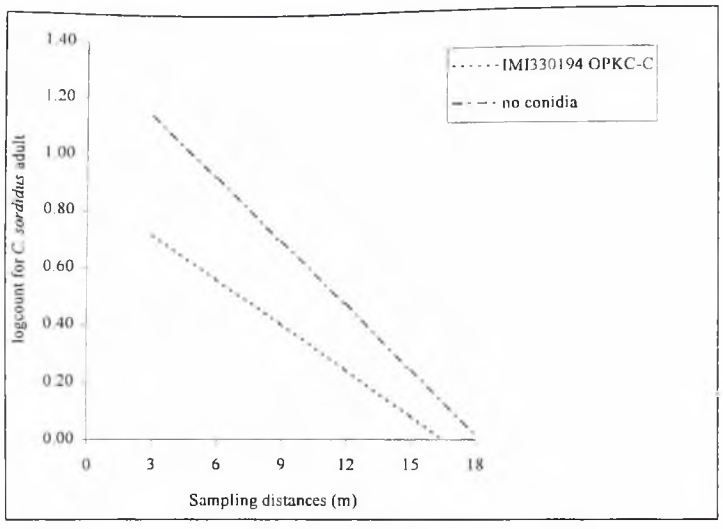

Figure 24. Regression fitted line for counts of *Cosmopolites sordidus* adults exposed or not exposed to OPKC-C of IMI330194 at different sampling distances recorded 2 to 63 days after release.

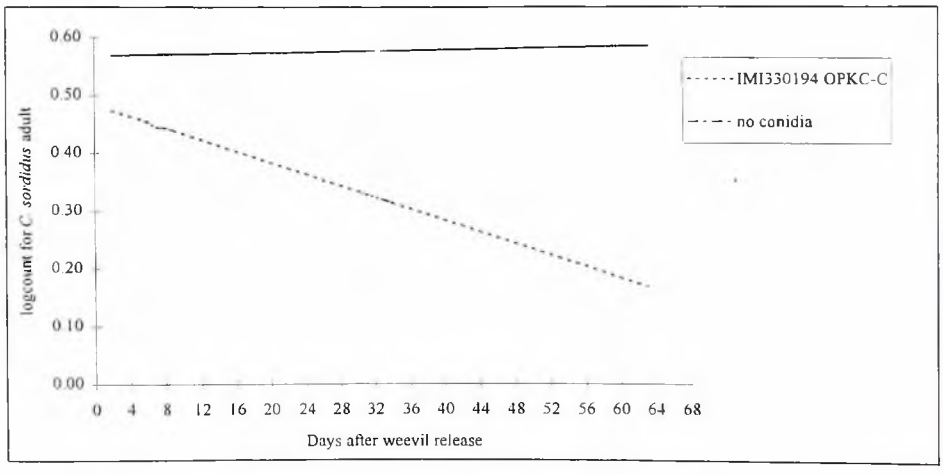

Figure 25. Regression fitted line for counts of *Cosmopolites sordidus* adults exposed or not exposed to OPKC-C of IMI330194 recorded from 2 to 63 days after weevil realease in a plantain field.

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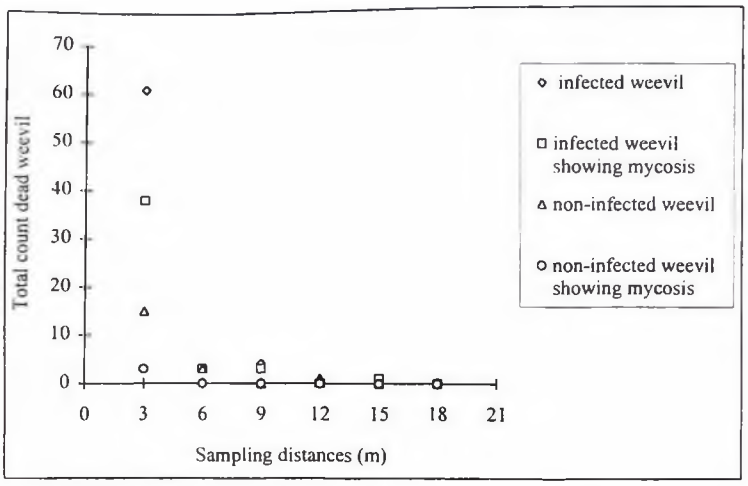

Figure 26. Mortality and sporulation of laboratory infected and non-infected *Cosmopolites sordidus* at different sampling distances 63 days after release in a plantain field.

6.4. DISCUSSION

The present study showed the potential of the strain IMI330194 for the management of *C. sordidus* adults in a field. Mortality of *C. sordidus* caused by the strain under laboratory and semi-field (pot experiments) conditions was similar to that under field conditions.

In general, mortality of *C. sordidus* exposed to the fungal conidia was higher (53.4-75.5%) than that of *C. sordidus* not exposed to the conidia (1.0-3.1%). Dead *C. sordidus* showed clear growth of *B. bassiana* when infected with OPKC-C and CP of IMI330194. The rate of mortality of *C. sordidus* obtained in this study was within the range reported by Batista-Filho *et al* (1995) in Brazil and Nankinga (1999) in Uganda. The mortality of *C. sordidus* and external growth of *B. bassiana* on dead weevils depended on the dose of conidia, the higher the dose of conidia the greater the effectiveness with respect to mortality and sporulation of *C. sordidus* (Table 21.). This result confirms similar findings reported by Nankinga (1994) and Busoli *et al* (1989) who showed that in the laboratory, increasing *B. bassiana* conidia dose increases the rate of mortality of *C. sordidus*.

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The infection of *C. sordidus* by conidia of *B. bassiana* did not prevent them from laying eggs. Larvae were collected both from treated and untreated suckers with *B. bassiana* strain IMI330194 conidia but the number of larvae in treated suckers was significantly less than that in untreated suckers. This could either have been fewer eggs were laid by the adult weevils exposed to treated suckers as a result of infection and death or eggs laid on treated suckers were infected and did not hatch. Nankinga (1994) reported that eggs and larvae of *C. sordidus* were susceptible to infection by *B. bassiana*. This observation was also supported by the previous results on egg and larval infection with *B. bassiana* strains in Chapter 4.

Coating suckers with CP or planting suckers with OPKC-C of IMI330194 gave the same degree of *C. sordidus* mortality (75.5%) when weevils were exposed to conidia immediately after application. Both OPKC-C of IMI330194 and CP of IMI330194 were effective in controlling the banana weevil under field conditions. A comparative trial of the two formulations in naturally weevil infested field showed that coating suckers with the CP of IMI330194 did not protect them from weevil attack. The suckers in this treatment suffered a similar level of attack (25.0%) compared to the control plants without conidia (33.3%). By contrast, few plants (5.6%) were attacked and none were killed with the OPKC-C of IMI330194 (Table 29.). One of the advantages of applying *B. bassiana* on OPKC was that the fungus continued to grow on the substrate, thus, the number of conidia increases in the delivery environment until the nutrient in the substrate is completely utilised or the substrate decayed. This resulted in enhancing the persistence of *B. bassiana* in the target habitat. The result obtained here supported that of Ogenga-Latigo and Masanza (1996). The same authors in their investigation on the effect of *B. bassiana* maize inoculum and chemical insecticides, reported a long-term effect of *B. bassiana* maize inoculum on *C. sordidus* adults.

Differences in results from the field where weevils were artificially released and the field naturally infested with weevils was, that in the former, weevils were immediately exposed to the conidia while in the latter, weevils may not have reached the treated

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plant immediately after conidia application. Therefore, the OPKC-C of IMI330194, being more persistent, had a greater advantage for killing the weevils over a longer period than the CP of IMI330194. The implication of this is that the suckers treated with OPKC-C of IMI330194 would have longer-term protection than suckers treated with CP of IMI330194. The sustained high mortality caused to *C. sordidus* by the OPKC-C of IMI330194 proved that it is a promising formulation for the management of adult weevils.

The study on the spread of *B. bassiana* in the plantain field by infected adult weevils revealed that the infected weevils moved less than 18 m (Figure 24.) within 60 days after infection and most of the infected weevil died at 3 m away from point of release (Figure 26.) and sporulated at this distance. The regression analysis for the count of weevils exposed and not exposed to *B. bassiana* formulation at the different sampling distances over the study period showed that the number of infected weevils found at the different sampling distances was significantly less than that of non-infected (Figure 24. and Appendix 35.). This is because the infected weevils died and their number decreased (Figure 26.). In the present study, after artificially infected and non-infected weevils were released, they became mixed together and of a total of 19 non-infected weevils died three showed signs of mycosis (Figure 26.). The implication of this result is that in a field situation, infected weevils can carry the disease to healthy ones. This is interesting as it may lead to an epizootic situation and reduce the weevil population.

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The present investigations addressed four main aspects, (i) a study of the duration of the different developmental stages of *C. sordidus* in the laboratory and their spatial distribution within plantain plants, (ii) selection of *B. bassiana* strains with respect to virulence and suitability for mass production, (iii) development and evaluation of different formulations of *B. bassiana* for the control of *C. sordidus* adults in pot experiments and, (iv) field evaluation of selected formulation for the management of the adult banana weevils.

Insight into the duration of *C. sordidus* developmental stages enabled appropriate sampling times to be selected for evaluation of the effect of *B. bassiana* on the stages and their spatial distribution on plantain. Similarly, the investigation on the location of the eggs, larvae and pupae of *C. sordidus* on the plantain plant guided the choice of where the formulation of *B. bassiana* should be applied against each stage. Knowledge of the location of *C. sordidus* within the plantain is a vital tool for efficient and economical application of *B. bassiana*. The eggs and larvae of *C. sordidus* were found both in the corm and pseudostem of plantain, while the pupae were only found in the corm. Thus the delivery of *B. bassiana* against the egg or larval stage must be directed to both the pseudostem and corm of plantain while that against the pupal and adult stage must be towards the corm from the collar of the plant or on the soil around the plant.

In the development of a mycoinsecticide, the selection of the most promising strains with respect to virulence on the insect is an important step. Bioassays in the laboratory clearly demonstrated that the virulence of the strains of *B. bassiana* on *C. sordidus* differs. The strains I94-907 and IMI330194 induced mycosis on adults of the banana

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weevil, while the strain ARSEF2624 did not. Similarly in previous studies Nankinga (1994) and Pena *et al.* (1993) reported differences in virulence of isolates of *B. bassiana* against *C. sordidus*.

The process of strain selection does not end after the stage of virulence testing. One must also consider the mass production potential. This is essential when the fungus needs to be delivered over a wide area. Entomopathogenic fungi such as *Beauveria* spp. can be produced on solid substrates. The substrates can be either nutritive, such as rice, bran, or wheat grains, or non-nutritive, such as vermiculite, sponge, or cloth (Jenkins and Goettel, 1997b). Studies showed the relative potential of the strains I94-907 and IMI330194 for conidial production on rice grains with the strain IMI330194 giving a higher number of conidia and good conidial viability. The strain IMI330194 was selected for further studies.

Once a pathogen strain is selected on the basis of its virulence and suitability for mass production, further steps need to be undertaken in the development of a mycopesticide. For this reason, a range of formulations of *B. bassiana* were developed and tested in pot experiments. High adult weevil mortality rates (> 60%) were obtained with GOK-CP, GO-CP, OPKC-C and CP of IMI330194. A clear advantage of the OPKC-C of IMI330194 was its ability to sustain high rates of mortality for at least 2 months after application.

Due to the promising results of *B. bassiana* strain IMI330194 against *C. sordidus* adults in the laboratory and pots experiments, trials were conducted in the field to test the efficacy of the fungus. OPKC-C of IMI330194 and CP of IMI330194 gave high levels of mortality (75.5%) when weevils were exposed to the fungus immediately after application (artificial infestation). Under natural weevil infestation, however, the CP of IMI330194 was not effective against the adult weevils. About 16.7% of plantain suckers treated with the CP of IMI330194 were killed while those treated with OPKC-C of IMI330194 remained alive. In conformity with the results from the pot experiments, the growth and viability of the fungus was sustained by the OPKC over the study period (60 days), potentially augmenting the number of conidia in the

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delivery environment until substrate nutrients were exhausted. The implication of this result is that OPKC-C of IMI330194 gives 2 months protection to suckers at a particularly susceptible stage in the plant's development. Long-term trials are needed to determine whether this initial protection translates into yield increase and longer plantation life.

The present study demonstrates clearly the potential for use of *B. bassiana* strain IMI330194 in the management of *C. sordidus*. Ultimately, biological control could form a component of an IPM strategy for weevils on plantain in Ghana together with cultural practices. Implementation will however, require adaptive research with respect to mass production, formulation and delivery.

The fungal production system is a technical one where equipment such as a Laminar flow cabinet, shaker and, autoclave were used. The first steps in the production system such, as isolation of the fungal pathogen from a dead insect, clean culture production, the liquid-phase of the two phase production system need close attention and use of aseptic methods to avoid contamination of the fungus being cultured. This may be a limiting factor to the production of *B. bassiana* at the farmers' level. The use of equipment such as Laminar flow cabinet and autoclave will undoubtedly increase the production cost and is therefore another limiting factor. Investigations should be done into reducing the costs of production by replacing or eliminating the advanced techniques with techniques that are appropriate for resource-poor farmers. For example the Laminar flow cabinet and the autoclave can be removed from the production system if a fungal strain that is less susceptible to contaminants is selected. The strain IMI330194 resisted invasion after application of three common contaminating fungi (*Aspergillus niger*, *Fusarium moniliforme* and *Penicillium hirsutum*) (Godonou *et al.*, 1999). This indicates that, the strain IMI330194 could be produced by simplifying the production system. Another limiting factor is the need for substrates for fungal production, which must be available at low cost. In the present studies, OPKC was obtained from sun-dried kernels using an oil extraction machine (a purposely designed press machine) and a corn mill machine. This procedure differs from the traditional method of extraction of oil in which the kernels are heated with

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wood fire. During the heating process the kernels turn black as a result of over-heating and the resulting cake is black. In the process of sun drying and the use of press machine, the cake is whitish with black spots (result of external shell of the kernel). The heating process in the traditional method of oil extraction may destroy some nutrients (e.g. proteins) in the resulting cake, which may reduce its ability to sustain the growth of *B. bassiana.* Therefore, its use in the present studies was ruled out. Nevertheless, studies might be initiated to test the traditionally made OPKC and other cheap substrates for the preparation of solid formulation of *B. bassiana* for the management of *C. sordidus*. Oil palm kernels are available in most of the growing areas of plantain in West Africa. OPKC may vary in chemical constitution depending on the method of oil extraction (traditional or industrial). The OPKC used for the fungal production were found to be cheap and available in most of the major plantain growing areas.

The OPKC-C of *B. bassiana* showed clearly that a solid carrier could enhance fungal persistence. Therefore a solid formulation may be the best to use against soil-borne insects such as *C. sordidus*.

Application of OPKC-C of *B. bassiana* strain IMI330194 to suckers in planting holes was successful. This technique is a novel approach compared to previous techniques of *B. bassiana* delivery against adult weevils, such as fungal application around mature plants or on plantain stumps (Nankinga, 1999; Gert Roland Fischer's Company COINBIOL-GRF, Guide for the use of *B. bassiana* to control Banana weevil in Brazil). However, results from field experiments showed that adult weevils deposit eggs on plantain suckers prior to death from *B. bassiana* infection. The resulting larval tunnels could ultimately lead to plant death. It is therefore recommended that delivery of the fungus should be towards weevils in crop residues in order to reduce the weevil population before they come into contact with plantain suckers. Crop residues could be assembled in the field at a spacing of not more than 18 m under which a solid formulation of *B. bassiana* such as OPKC-C of *B. bassiana* would be applied. This strategy would have the advantage of reducing the amount of

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fungal formulation to be applied per hectare compared to the technique of treating individual plants. Also this technique would be less labour intensive.

In conclusion, *B. bassiana* strain IMI330194 has shown considerable promise as a highly virulent microbial agent for use against banana weevils on plantain. In particular, the strain mass-produced, formulated on OPKC and applied in planting holes provided a sustained protection to plantain suckers at the early stage of the plants development especially vulnerable to *C. sordidus*. OPKC was found to sustain the growth of *B. bassiana* strain IMI330194 in planting holes and may also have acted as plant fertiliser in view of the kernel's chemical composition express in percent dry matter (Crude protein: 11.9; crude fibre: 31.6; Nitrogen free extract: 25.9; Ether extract: 26.9) (Ocampo, 1994). It was observed that suckers planted with OPKC-C of *B. bassiana* have showed vigorous growth compared to those planted without OPKC-C and this aspect can help young plantain suckers to survive weevil damage. The current technology for the management of banana adult weevils, which represents an advance compared with previous research, needs further testing prior to implementation. The challenge for the future will be the development of a delivery system taking into account the biology and the cryptic habit of the weevil. For this reason further research may look at the following:

- Production and formulation of the strain IMI330194 of *B. bassiana* on the traditionally made and available OPKC in comparison with the industrial one that was used in the present study. If the production is not so good on the traditionally made OPKC compare to the industrially made, it must be amended with substrates which can provide nitrogen and carbohydrate which are the major nutrients that affect the growth of enthomopathogens such *as B. bassinet*.

- Study the fertiliser effect of OPKC on the growth of plantain which effect can probably contribute to the vigorous growth of plantain suckers and therefore, help the plants to survive *C. sordidus* attack.

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- Screen for more locally available, waste or cheap solid substrate for the production and formulation of the strain IMI330194.

- Studies on the cost effectiveness for using OPKC-C for the management of *C. sordidus*. The studies must look at the production cost including packaging and storage.

- Integrate the use of OPKC-C or alternative solid formulation of the strain IMI330194 of *B. bassiana* with already existing control measures such as cultural (weeding and removal of all refuse from the plantain field, and use of harvested pseudostem and rhizome as traps), chemical control (use of pheromone for trapping and killing) and biological control (use of entomopathogenic nematodes, predators). In addition to protecting the sucker with OPKC-C, studies should be carried out on the use of harvested pseudostem and rhizome that can be bulked into patches within or at the edges of the weeded and clean plantain field and the OPKC-C applied underneath. The harvested materials will serve as a trap for banana weevils and favour the weevil contact with the OPKC-C. In the same way the OPKC-C can be used in combination with a chemical attractant of *C. sordidus* e.g. pheromone or plantain extract (semio-chemical: kairomone). The weevils that are attracted to the OPKC-C will be undoubtedly infected and do not need to be collected and killed. They can be left in the field as a source of inoculum for infecting the remaining healthy weevils.

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APPENDICES

Appendix 1. Likelihood Ratio Statistics For Type 1 Analysis for the number of eggs of *C. sordidus* at three levels on growing plantain.

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	105.7405	0		
Sampling levels	100.2237	2	5.5167	0.0634

Appendix 2. Likelihood Ratio Statistics For Type 1 Analysis for number of larvae at three levels on growing plantain.

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	93.2614	0		
Sampling level	80.5744	2	12.6870	0.0018

Appendix 3. Miscellaneous Techniques for spore counting using an improved Neubauer haemacytometer

It is essential to know the spore concentration of all formulations to be used in the laboratory or in the field. The concentration of a spore suspension can be determined using a haemacytometer.

a/ Diluting formulated spores in water plus 0.05% Tween 80 or in oil

In the present study spores were suspended in sterile water plus 0.05% Tween 80. For counting, the ideal concentration of a conidial suspension is between $1x10^6$ and $1x10^7$ conidia/ml. A conidial suspension of *Beauveria* spp. will appear light whitish when suspended in water with 0.05% Tween 80. Conidial suspensions which are more concentrated than this will need to be diluted before counting.

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1 Shake the bottle containing the formulated spores to ensure that the spores are single distributed in the solution .

2 Take 1 ml. of the formulation and add this to 9 ml. of water with 0.05% Tween 80

3 If the solution looks too concentrated (too white), shake the suspension well and re-dilute 1 ml. of this solution with a further 9 ml. of water with 0.05% Tween 80.

N.B. Be as accurate and exact as possible when preparing the 9 ml. of water plus 0.05% Tween 80 and taking the sample of concentrated spore suspension. If these volumes are not measured accurately, your results will be meaningless.

b/ Preparing the haemacytometer

The haemacytometer must be dry and free of grease such as finger prints etc. Grease should be removed using a tissue and alcohol.

1 Using clean finger wipe some saliva over the two edge of the cover slip

2 Place it on the haemacytometer and apply a little pressure so that you can see a rainbow (Newton's rings) at both edges of the slide.

3 Use a Pasteur pipette to take a small amount of the diluted solution.

4 Drop the solution at the edge of the slip, it will be drawn up under the cover slip. Do not overfill the chamber, the channels (grooves) at the sides of the chamber are for taking up any excess solution only and should not be filled with liquid.

5 Leave for few minutes until the spores have settled (5 minutes)

c/ Counting of spores

1 Under the microscope you can see a gird as shown in Appendix 4.

2 If the concentration is very low count all 25 squares.

3 If the concentration is higher count 5 squares on the diagonal (See highlighted squares, Appendix 4.)

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4 One large square is made up of 16 smaller squares and is bordered by 3 lines. There are 25 large squares on each grid of an improved Neubauer haemacytometer.

d/ Calculation of concentration of spore

If 5 large squares were to be counted

1 Count the number of spores in each of 5 large squares.

2 Add up the total number of spores counted in 5 squares

3 Repeat this count on a second grid as indicated above.

4 To calculate the mean spore count:

Let a and b = totals for each grid

Let X = mean count of 5 squares

$$X = \frac{(a + b)}{2}$$

5 You used 0.1µl of spore suspension over 25 squares

Let c = concentration of spores per ml

$c = X \times 5 \times 10^4$

6 Let n the number of dilution and

C = the concentration of spores in the original solution

$C = c \times 10^n$

If 25 large squares were to be counted

1 Count the number of spores in each grid.

2 Add up the total number of spores counted in 25 squares

3 To calculate the mean spore count:

Let a and b = totals for each grid

Let X = mean count of 25 squares

$$X = \frac{(a + b)}{2}$$

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4 You used 0.1µl of spore suspension over 25 squares

Let c = concentration of spores per ml

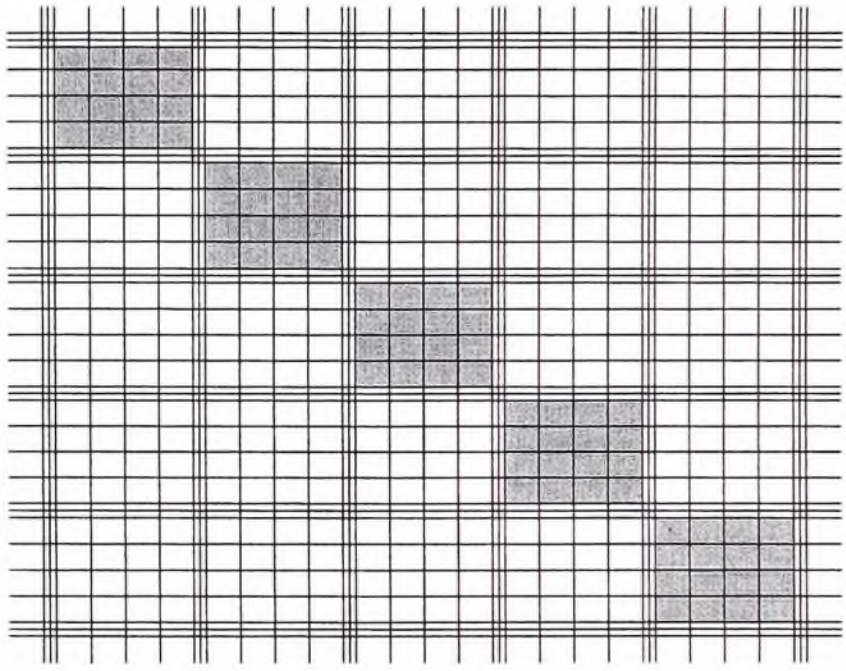
$c = X \times 10^4$

5 Let n the number of dilution and

Let C = the concentration of spores in the original solution

$C = c \times 10^n$

Appendix 4. Grid on an improved Neubauer haemacytometer



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Appendix 5. General Linear Model (GLM) for percentage cumulative mortality of *C. sordidus* adults at days 7, 14 and 21 after exposure to two delivery materials (pseudostem traps and pieces of rhizome) treated with inoculum of *B. bassiana* strains ARSEF2624 or I94-907.

a) GLM (ANOVA Table) for percentage cumulative mortality of *C. sordidus* at day 7

source of variation	df	Sum of Square (SS)	Mean of Square (MS)	F	P > F
Material	1	24.31	24.31	1.05	0.4141
Strain	2	563.05	281.53	12.10	0.0763
Material x Strain	2	46.52	23.26	0.30	0.7455
Error	21	1639.61	78.08		
Total	26	2273.49			

b) ANOVA Table for percentage cumulative mortality of *C. sordidus* at day 14

source of variation	df	SS	MS	F	P >F
Material	1	8.51	8.51	0.22	0.6871
Strain	2	2300.65	1150.33	29.35	0.0330
Material x Strain	2	78.39	39.20	0.49	0.6189
Error	21	1676.82	79.85		
Total	26	4064.37			

c) ANOVA Table for percentage cumulative mortality of *C. sordidus* at day 21

source of variation	df	SS	MS	F	P >F
Material	1	11.57	11.57	0.27	0.6530
Strain	2	3054.97	1527.48	36.14	0.0269
Material x Strain	2	84.53	42.27	0.58	0.5686
Error	21	1530.14	72.86		
Total	26	4681.21			

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Appendix 6. GLM for percentage cumulative dead weevils with signs of mycosis at days 7, 14 and 21 after exposure to two delivery materials (pseudostem traps and pieces of rhizome) treated with inoculum of *B. bassiana* strains ARSEF2624 or I94-907.

a) ANOVA Table for percentage cumulative dead weevils with signs of mycosis at day 7

source of variation	df	SS	MS	F	P >F
Material	1	124.70	124.70	1.02	0.4190
Strain	2	244.67	122.34	1.00	0.5000
Material x Strain	2	244.49	122.34	7.21	0.0041
Error	21	356.49	16.98		
Total	26	970.53			

b) ANOVA Table for percentage cumulative dead weevils with signs of mycosis at day 14

source of variation	df	SS	MS	F	P >F
Material	1	5.40	5.40	0.91	0.4399
Strain	2	3415.01	1707.50	288.97	0.0034
Material x Strain	2	11.82	5.91	0.28	0.7595
Error	21	445.20	21.20		
Total	26	3877.43			

c) ANOVA Table for percentage cumulative dead weevils with signs of mycosis at day 21

source of variation	df	SS	MS	F	P >F
Material	1	4.16	4.16	0.90	0.4423
Strain	2	3682.29	1841.15	399.95	0.0025
Material x Strain	2	9.21	4.60	0.24	0.7893
Error	21	404.05	19.24		
Total	26	4099.71			

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Appendix 7. ANOVA for number of conidia produced by the strains I94-907 and IMI330194 of *B. bassiana* on rice grains

Source of variation	df	SS	MS	F	P>F
Strain	1	0.0144	0.0144	2.07	0.2234
Error	4	0.0277	0.0069		
Total	5	0.0421			

Appendix 8. ANOVA for weight of conidial powder of the strains I94-907 and IMI330194 of *B. bassiana* produced on rice grains

Source of variation	df	SS	MS	F	P>F
Strain	1	0.3698	0.3698	2.88	0.1407
Error	6	0.7710	0.1285		
Total	7	1.1408			

Appendix 9. ANOVA for number of conidia of the strains I94-907 and IMI330194 of *B. bassiana* in extracted conidial powder from rice grains

Source of variation	df	SS	MS	F	P>F
Strain	1	0.0368	0.0368	1.32	0.2942
Error	6	0.1670	0.0278		
Total	7	0.2038			

Appendix 10. ANOVA for viability of conidia of the strains of *B. bassiana* I94-907 and IMI330194 on rice grains

Source of variation	df	SS	MS	F	P>F
Strain	1	0.2204	0.2204	236.16	0.0001
Error	4	0.0037	0.0009		
Total	5	0.2241			

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Appendix 11. ANOVA for viability of conidia in conidial powder of the strains I94-907 and IMI330194 of *B. bassiana* extracted form rice grains

Source of variation	df	SS	MS	F	P>F
Strain	1	0.5046	0.5046	22.00	0.0094
Error	4	0.0917	0.0229		
Total	5	0.5963			

Appendix 12. ANOVA for percentage cumulative mortality of *C. sordidus* adults at days 7, 14 and 21 after exposure to *B. bassiana* strains I94-907 and IMI330194

a) ANOVA Table for percentage cumulative mortality at day 7

Source of variation	df	SS	MS	F	P > F
Strain	2	1088.91	544.46	9.05	0.0070
Error	9	541.68	60.19		
Total	11	1630.59			

b) ANOVA Table for percentage cumulative mortality at day 14

Source of variation	df	SS	MS	F	P > F
Strain	2	2648.84	1324.42	21.50	0.0004
Error	9	554.29	61.59		
Total	11	3203.13			

c) ANOVA Table for cumulative percentage mortality at day 21

Source of variation	df	SS	MS	F	P > F
Strain	2	2917.42	1458.71	21.25	0.0004
Error	9	617.90	68.66		
Total	11	3535.32			

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Appendix 13. ANOVA for percentage cumulative dead adults of *C. sordidus* with signs of mycosis at days 7, 14 and 21 after exposure to *B. bassiana* strains I94-907 and IMI330194

a) ANOVA Table for percentage cumulative dead adults of *C. sordidus* with signs of mycosis at days 7

Source of variation	df	SS	MS	F	P > F
Strain	2	408.37	204.19	2.96	0.1028
Error	9	620.87	68.99		
Total	11	1029.24			

b) ANOVA Table for percentage cumulative dead adults of *C. sordidus* with signs of mycosis at days 14

Source of variation	df	SS	MS	F	P > F
Strain	2	2820.30	1410.15	23.83	0.0003
Error	9	532.66	59.18		
Total	11	3352.96			

c) ANOVA Table for percentage cumulative dead adults of *C. sordidus* with signs of mycosis at days 21

Source of variation	df	SS	MS	F	P > F
Strain	2	3853.95	1926.97	22.54	0.0003
Error	9	769.33	85.4815		
Total	11	4623.28			

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Appendix 14. ANOVA for percentage cumulative non-hatched eggs of *C. sordidus*, 10 days after exposure to pieces of corm treated with inoculum of *B. bassiana* strain I94-907.

Source of variation	df	SS	MS	F	P > F
Strain	1	93.21	93.21	0.55	0.4808
Error	8	1363.93	170.49		
Total	9	1457.14			

Appendix 15. ANOVA for percentage cumulative non-hatched eggs of *C. sordidus* with signs of mycosis, 10 days after exposure to pieces of corm treated with inoculum of *B. bassiana* strain I94-907.

Source of variation	df	SS	MS	F	P > F
Strain	1	1675.73	1675.73	10.15	0.0129
Error	8	1320.25	165.03		
Total	9	2995.98			

Appendix 16. ANOVA for percentage cumulative non-hatched egg of *C. sordidus*, 10 days after exposure to pieces of corm treated with inoculum of *B. bassiana* strain IMI330194.

Source of variation	df	SS	MS	F	P > F
Strain	1	1773.40	1773.40	9.43	0.0219
Error	6	1128.31	188.05		
Total	7	2901.71			

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Appendix 17. ANOVA for percentage cumulative non-hatched egg of *C. sordidus* with signs of mycosis, 10 days after exposure to pieces of corm treated with inoculum of *B. bassiana* strain IMI330194.

Source of variation	df	SS	MS	F	P > F
Strain	1	1424.71	1424.71	18.74	0.0049
Error	6	456.08	76.01		
Total	7	1880.79			

Appendix 18. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 7 days after treatment with I94-907 inoculum

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	53.4018	0		
Inoculum	44.2549	1	9.1469	0.0025

Appendix 19. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 21 days after treatment with inoculum of *B. bassiana* strain I94-907

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	60.2838	0		
Inoculum	52.2999	1	7.9839	0.0047

Appendix 20. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 7 days after treatment with inoculum of *B. bassiana* strain IMI330194

Source	Deviance	DF	chi-square	Pr>Chi
INTERCEPT	52.9251	0		
Inoculum	50.2193	1	2.7058	0.1000

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Appendix 17. ANOVA for percentage cumulative non-hatched egg of *C. sordidus* with signs of mycosis, 10 days after exposure to pieces of corm treated with inoculum of *B. bassiana* strain IMI330194.

Source of variation	df	SS	MS	F	P > F
Strain	1	1424.71	1424.71	18.74	0.0049
Error	6	456.08	76.01		
Total	7	1880.79			

Appendix 18. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 7 days after treatment with I94-907 inoculum

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	53.4018	0		
Inoculum	44.2549	1	9.1469	0.0025

Appendix 19. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 21 days after treatment with inoculum of *B. bassiana* strain I94-907

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	60.2838	0		
Inoculum	52.2999	1	7.9839	0.0047

Appendix 20. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 7 days after treatment with inoculum of *B. bassiana* strain IMI330194

Source	Deviance	DF	chi-square	Pr>Chi
INTERCEPT	52.9251	0		
Inoculum	50.2193	1	2.7058	0.1000

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Appendix 21. Likelihood Ratio Statistics For Type 1 Analysis for proportion of dead larvae of *C. sordidus* within pieces of corm 21 days after treatment with inoculum of *B. bassiana* strain IMI330194

Source	Deviance	df	chi-square	Pr>Chi
INTERCEPT	55.0511	0		
Inoculum	51.3550	1	3.6961	0.0545

Appendix 22. ANOVA for weight of *B. bassiana* strain IMI330194 conidial powder produced on rice grains subjected to three treatments

source of variation	df	SS	MS	F	P >F
Treatment	2	5.97	2.98	11.14	0.0037
Error	9	2.41	0.27		
Total	11	8.38			

Appendix 23. ANOVA for number of *B. bassiana* strain IMI330194 conidia produced on rice grains subjected to three treatments

source of variation	df	SS	MS	F	P >F
Treatment	2	0.0028	0.0014	0.19	0.8328
Error	6	0.0447	0.0075		
Total	8	0.0476			

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Appendix 24. ANOVA for viability of conidia of *B. bassiana* strain IMI330194 produced on rice grains subjected to three treatments

source of variation	df	SS	MS	F	P >F
Treatment	2	0.0017	0.0008	0.60	0.5787
Error	6	0.0083	0.0014		
Total	8	0.0100			

Appendix 25. ANOVA for number of conidia of *B. bassiana* strain IMI330194 produced on oil palm kernel cake and rice grains at different moisture content

source of variation	df	SS	MS	F	P >F
Treatment	9	11.44	1.27	45.35	0.0001
Error	20	0.56	0.03		
Total	29	12.00			

Appendix 26. ANOVA for viability of conidia of the strain IMI330194 produced on on oil palm kernel cake and rice grains at different moisture content

source of variation	df	SS	MS	F	P >F
Treatment	9	1.02	0.1131	9.50	0.0001
Error	20	0.24	0.0119		
Total	29	1.26			

Appendix 27. GLM for percentage cumulative mortality of *C. sordidus* and percentage cumulative dead *C. sordidus* with signs of mycosis at days 26 and 42 after exposure to W-CP and GOK-CP of *B. bassiana* strain IMI330194

a) ANOVA for percentage cumulative mortality of *C. sordidus* at day 26 after exposure

source of variation	df	SS	MS	F	P >F
Formulation	5	7419.08	1483.82	14.74	0.0001
Error	17	1711.04	100.65		
Total	22	9130.12			

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b) ANOVA for percentage cumulative mortality of *C. sordidus* at day 42 after exposure

source of variation	df	SS	MS	F	P >F
Formulation	5	7225.92	1445.18	11.53	0.0001
Error	17	2130.91	125.35		
Total	22	9356.83			

c) ANOVA for percentage cumulative dead adults with signs of mycosis at day 26 after exposure

source of variation	df	SS	MS	F	P >F
Formulation	5	7147.40	1429.48	20.73	0.0001
Error	17	1172.47	68.97		
Total	22	8319.87			

d) ANOVA for percentage cumulative dead adults with signs of mycosis at day 42 after exposure

source of variation	df	SS	MS	F	P >F
Formulation	5	8124.22	1624.84	25.84	0.0001
Error	17	1068.87	62.87		
Total	22	9193.09			

Appendix 28. ANOVA for percentage cumulative mortality and percentage cumulative dead *C. sordidus* showing signs of mycosis at 18 days after exposure to GO-CP and CS-CP of *B.bassiana* strain IMI330194

a) ANOVA Table for percentage cumulative mortality of *C. sordidus*

source of variation	df	SS	MS	F	P >F
Formulation	5	7183.88	1436.78	17.55	0.0001
Error	12	982.58	81.88		
Total	17	8166.46			

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b) ANOVA Table for percentage cumulative dead adults of *C. sordidus* showing signs of mycosis

source of variation	df	SS	MS	F	P >F
Formulation	5	4887.23	977.44	40.50	0.0001
Error	12	289.60	24.13		
Total	17	5176.83			

Appendix 29. ANOVA for percentage cumulative mortality and percentage cumulative dead adults of *C. sordidus* showing signs of mycosis 18 and 35 days after exposure to OPKC-C of *B. bassiana* strain IMI330194

a) ANOVA for percentage cumulative mortality of *C. sordidus* 18 days after exposure

source of variation	df	SS	MS	F	P >F
Formulation	2	4160.72	2080.36	40.13	0.0001
Error	8	414.76	51.84		
Total	10	4575.48			

b) ANOVA for percentage cumulative mortality of *C. sordidus* 35 days after exposure

source of variation	df	SS	MS	F	P >F
Formulation	2	6487.03	3243.51	14.79	0.0021
Error	8	1754.08	219.26		
Total	10	8241.11			

c) ANOVA for percentage cumulative dead adults of *C. sordidus* showing signs of mycosis 18 days after exposure

source of variation	df	SS	MS	F	P >F
Formulation	2	2796.23	1398.12	25.60	0.0003
Error	8	436.84	54.60		
Total	10	3233.07			

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d) ANOVA for percentage cumulative dead adults of *C. sordidus* showing signs of mycosis 35 days after exposure

source of variation	df	SS	MS	F	P >F
Formulation	2	5760.13	2880.06	48.32	0.0001
Error	8	476.84	59.60		
Total	10	6236.97			

Appendix 30. GLM for percentage cumulative mortality and cumulative percentage dead adults of *C. sordidus* with signs of mycosis after exposure to IMI330194 formulations at day 0, 14, 28 and 42 after sucker treatment.

a) GLM (ANOVA Table) for percentage cumulative mortality of *C. sordidus*

source of variation	df	SS	MS	F	P >F
DAY	3	8412.67	2804.22	32.27	0.0001
FORM	3	13337.67	4445.89	51.16	0.0001
DAY*FORM	9	4294.00	477.11	5.49	0.0001
Error	48	4171.18	86.90		
Total	63	30215.52			

b) GLM (ANOVA Table) for percentage cumulative dead adults of *C. sordidus* with signs of mycosis

source of variation	df	SS	MS	F	P >F
DAY	3	3666.76	1222.25	23.01	0.0001
FORM	3	8184.35	2728.12	51.36	0.0001
DAY*FORM	9	1837.80	204.20	3.84	0.0010
Error	48	2549.73	53.12		
Total	63	16238.65			

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Appendix 31. ANOVA for percentage cumulative weevil mortality and percentage dead of *C. sordidus* with signs of mycosis, 28 days after exposure to two doses of *B. bassiana* conidia in a field.

a) ANOVA for percentage cumulative weevil mortality

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	1058.82	529.41	1.91	0.2613
Dose (D)	2	41988.16	20994.08	75.83	0.0007
Bl*D	4	1107.41	276.85		
Error	99	26656.50	269.26		
Total	107	70810.89			

b) ANOVA for percentage cumulative dead of *C. sordidus* with signs of mycosis

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	287.99	143.99	0.92	0.4693
Dose (D)	2	42089.00	21044.50	134.39	0.0002
Bl*D	4	626.36	156.59		
Error	99	21757.61	219.77		
Total	107	64760.96			

Appendix 32. ANOVA for percentage cumulative weevil mortality and percentage dead of *C. sordidus* with signs of mycosis in planting holes, 28 days after exposure to two doses of *B. bassiana* strain IMI330194 conidia in a field.

a) ANOVA for percentage cumulative weevil mortality in the planting holes

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	1549.20	774.60	1.75	0.2841
Dose (D)	2	19068.62	9534.31	21.57	0.0072
Bl*D	4	1767.90	441.97		
Error	99	22306.33			
Total	107	44692.05			

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b) ANOVA for percentage dead *C. sordidus* with signs of mycosis in the planting holes

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	1488.86	744.43	2.31	0.2155
Dose (D)	2	15666.28	7833.14	24.29	0.0058
Bl*D	4	1289.85	322.46		
Error	99	18621.94	188.10		
Total	107	37066.93			

Appendix 33. ANOVA for percentage cumulative weevil mortality and percentage dead *C. sordidus* with signs of mycosis, 28 days after exposure to OPCK-C of *B. bassiana* strain IMI330194 in a field

a) ANOVA for percentage cumulative weevil mortality

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	639.01	319.50	0.83	0.4987
Formulation (F)	2	71534.01	35767.00	93.16	0.0004
Bl*F	4	1535.71	383.93		
Error	99	20679.83	208.89		
Total	107	94388.56			

b) ANOVA for percentage dead *C. sordidus* with signs of mycosis

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	158.63	79.31	2.73	0.1790
Formulation (F)	2	34607.32	17303.66	595.05	0.0001
Bl*F	4	116.31	29.08		
Error	99	14803.19	149.53		
Total	107	49685.45			

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Appendix 34. ANOVA for percentage cumulative weevil mortality and Percentage dead a *C. sordidus* with signs of mycosis in planting holes, 28 days after exposure to OPKC-C of *B. bassiana* strain IMI330194 in a field.

a) ANOVA for percentage cumulative weevil mortality in planting holes

source of variation	DF	SS	MS	F	Pr > F
Block (Bl)	2	1104.17	552.09	2.08	0.2400
Formulation (F)	2	21807.88	10903.94	41.13	0.0022
Bl*F	4	1060.43	265.11		
Error	99	19759.33	199.59		
Total	107	43731.81			

b) ANOVA for percentage dead *C. sordidus* with signs of mycosis in planting holes

source of variation	df	SS	MS	F	P > F
Block (Bl)	2	624.27	312.13	1.59	0.3103
Formulation (F)	2	7941.00	3970.50	20.23	0.0081
Bl*F	4	785.05	196.26		
Error	99	15687.37	158.46		
Total	107	25037.69			

Appendix 35. Regression analysis for counts of *C. sordidus* adults exposed and not exposed to OPKC-C of *B. bassiana* strain IMI330194 after release in a plantain field at different sampling distances and over the study period.

Parameter	Estimate[+]	SE	t (738)	t probability
Constant	1.0433	0.0401	26.05	<.001
days	-0.005009	0.000768	-6.52	<.001
Distance	-0.05325	0.00268	-19.86	<.001
Treatment	0.3137	0.0566	5.54	<.001
Days*Treatment	0.00528	0.00109	4.86	<.001
Distance*Treatment	-0.02178	0.00379	-5.74	<.001

+ Estimate in differences in slopes of the regression lines for the counts of *C. sordidus* exposed and unexposed to IMI330194 conidia.