

***In vitro* pathogenicity evaluation of South African and exotic strains of *Beauveria bassiana* against two Coleopteran storage pests: *Sitophilus zeamais* (Motschulsky) and *Lasioderma serricorne* (Fabricius)**

Masiyiwa Ngoni Sakupwanyanya

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Masters of Science.

Johannesburg, 2007

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the Degree of Master of Science to the University of the Witwatersrand, Johannesburg. It has not been submitted to any other university for any other purposes or examination.

Masiyiwa Ngoni Sakupwanya

_____ day of _____ 2007

ABSTRACT

Entomopathogenic fungi are ubiquitous, soil borne microorganisms of scientific importance owing to their potential use as biological control agents of a wide range of arthropod pests of agricultural produce. The aims of this study included an assessment of this potential against two prominent insect pests of stored agro-commodities under controlled laboratory conditions, which presented the opportunity to isolate strains indigenous to South Africa. Three local strains of the entomopathogen, *Beauveria bassiana*, were successfully isolated from soils in the Northern Province and in comparative bioassays with five exotic strains of the same fungus evaluated to:

1. Determine the effects of different temperature on vegetative growth of the fungus.
2. Determine the effects of *B. bassiana* against the maize weevil, *Sitophilus zeamais* (Motschulsky) (Coleoptera) using water formulations and dry spores to infect the insect.
3. Determine the effects of *B. bassiana* against the tobacco beetle, *Lasioderma serricornis* (Fabricius) (Coleoptera) using water formulations at inundative rates of 10^8 conidia ml^{-1} to infect the pest.

Results indicated that the growth of local isolates of *B. bassiana* at different temperature was comparable with that of exotic isolates. Most isolates were found to grow optimally at 25 °C, with two isolates growing optimally at 20 °C and one at 28 °C. *In vitro* evaluations of the effects of *B. bassiana* against the maize weevil results included inundative rate inoculations, dose response assessments, effects of various temperatures on fungal virulence and dry spore assessments. In preliminary bioassays assessing the pathogenicity of *B. bassiana* against the maize weevil, insects were infected using water formulations of the fungus at inundative rates of 10^8 conidia ml^{-1} . The insect was highly susceptible to infection, with an average of 92% mortality obtained for the eight isolates tested. Local isolates conferred mortality levels that were equal to and better than exotic isolates, highlighting that native strains of *B. bassiana* can be used to equal effect against the maize weevil as exotic strains. Tests to confirm the occurrence of an entomopathogenic infection through the stimulation of mycosis resulted in all fungus-inoculated insects being mummified by fungal growth and scanning electron micrographs are presented showing this growth through insect appendages. For evaluation of fungal

virulence at different conidial concentrations, a local isolate (PPRI 04307) was selected and mortality levels of between 10 and 100% were obtained using conidial concentrations that ranged from 10^3 to 10^8 conidia ml^{-1} . Results indicated that insect mortality levels were dependent on conidial concentration, and rose with an increase in conidia within the inoculum. The lethal dose of *B. bassiana* to kill 50% of inoculated maize weevils was established to be 10^6 conidia ml^{-1} . To evaluate fungal pathogenicity under different environmental conditions using temperature as the main parameter, a local isolate (PPRI 04306) was used. Fungal virulence was ultimately retarded at 15 °C and 37 °C, however results indicate a clear pattern in both mortality and mycosis of an increase from 15°C to a maximum at 25 °C followed by a decrease at 30 °C and 37 °C. Further assessments used *Beauveria bassiana*-mycosed cadavers as carriers to deliver or transmit infectious dry fungal spores to live insects with no added moisture. Mortality levels were significantly higher in fungus-treated plots compared with fungus-free plots, and ranged from between 73 to 90% for the three isolates evaluated which included one exotic (IMI386 701) and two local (PPRI 04306 and PPRI 04307) isolates. Inundative conidial concentrations (10^8 conidia ml^{-1}) of *B. bassiana* water formulations were also used to infect the tobacco beetle and results showed that the insect was highly susceptible to infection by the fungus with mortality levels of between 85 and 100% obtained using both local and exotic isolates. This is a first account reporting this insect-pathogen interaction *in vitro*. Light and scanning electron microscopy images are presented of the resultant mycosis from fungus-treated cadavers showing the emergence of spores and mycelia from within the insect. This study establishes that the entomopathogen *B. bassiana* can be harnessed locally for use in the development of bioinsecticides, provides base-line data of its effects against the two afore mentioned pests and provides further evidence of the wide host range and ubiquitous nature of *B. bassiana*.

ACKNOWLEDGMENTS

The National Research Foundation of South Africa is gratefully acknowledged for the financial assistance provided for this study. My sincere thanks go to my academic supervisors Professor Colin Straker and Professor Vince Gray who provided me with the opportunity to conduct this study under their diligent guidance. Further thanks are given to my colleagues in the Biological Control Team, Dr. L. Ngoma, Dr. M. Jumba, Miss. N. Morar and Miss M. Nyamboli, and to the School of Molecular and Cell Biology, whose facilities were used during this study. Many thanks are also given to the support staff within the school. I deeply appreciate the advice and assistance given to me by Dr. Mariena Aquino De Muro from CABI Biosciences in England, who provided me with fungal cultures free of charge. The assistance I received in the provision of live insect cultures from Mr. Frikkie Kirsten and his team at the Stored Grain and Oil Seed Research Unit, Plant Protection Research Institute in Roodeplaat, South Africa, is also deeply appreciated. I am grateful for the assistance given to me by Caroline Lalkhan and Abe Seema of the Electron Microscopy Unit as well as Mr. V. Schneiderman of APES for allowing me to use their growth rooms. I am especially grateful, thankful and indebted to my friends here at the Witwatersrand (Tatenda Gatawa, Morgen Mutsau, Thabang Ntho, Grace Musila, James Moyo, Edwin and Kagiso Mmutlane, Lorenzo Giovanelli, Roy Shiri, Masimba Murugweni, Luck Sinwell, Patricia Mutsvunguma, Irene Ketseoglou, Kenneth Mabasa, Kennedy Kotwombe and the late, great Mr. Sam Farai Mugabe) and to my family whose continual support and encouragement helped me throughout this project and my time at university. Finally I thank my external scientists: Dr. Dave Moore for his mentorship and visit to the Mycological Research Laboratory at here Wits, and Dr. Jeff Lord for his input through correspondence.

TABLE OF CONTENTS

DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF FIGURES	ix
LIST OF TABLES	xiii
LIST OF ABBREVIATIONS	xv

CHAPTER ONE – Introductory and Literature Review

1.1	General Introduction and Project Objectives	1
1.2	Literature Review	6
1.2.1	Biological Control	6
1.2.2	Strategies of Biological Control	7
1.2.3	Overview of Biological Control of Insects	8
1.3	Insect Pests	15
1.3.1	Pests with emphasis on pest of stored products	15
1.3.2	Insect pest taxonomic classification and biology	17
1.3.3	Conventional control measure for storage pests	23
1.3.4	Resistance to insecticides	26
1.4	Entomopathogenic Fungi (EPF)	26
1.4.1	Taxonomic distribution and description of EPF	26
1.4.2	Biology and mode of action of EPF	28
1.4.3	EPF as biological control agents	32
1.5	Notes on and design of the present study	41

CHAPTER TWO – Establishment of Fungal Cultures

2.1	Sourcing of Fungal Isolates	44
2.1.1	Introduction	44
2.2	Materials and Methods	47
2.2.1	Sourcing of indigenous isolates through use of the <i>Galleria</i> bait method	47
2.2.2	Maintenance of fungal cultures	51

CHAPTER THREE – Thermal Tolerance Evaluation

3.1	Experiment to Determine the Effects of Varying Temperatures on Vegetative Radial Growth	53
3.1.1	Introduction	53
3.1.2	Materials & methods	54
3.1.3	Data Analysis	55
3.2	Results and Discussion	55

CHAPTER FOUR – Pathogenicity of *Beauveria bassiana* against the Maize Weevil (*Sitophilus zeamais*)

4.1	Introduction	59
4.2	Materials and Methods	60
4.2.1	Maintenance and cultures of experimental organisms	60
4.2.2	Preliminary bioassays	63
4.2.3	Scanning electron microscopy (SEM)	64
4.2.4	Effects of different conidial concentrations on fungal virulence	65
4.2.5	Effects of temperatures on fungal virulence	66
4.2.6	Ability of dry spores from cadavers to infect live weevils	67
4.2.7	Data Analysis	68
4.3	Results and Discussion	69
4.3.1	Preliminary bioassays	69
4.3.2	Scanning electron microscopy (SEM)	72
4.3.3	Effects of different conidial concentrations on fungal virulence	73
4.3.4	Effects of temperatures on fungal virulence	75
4.7.4	Ability of dry spores from cadavers to infect live weevils	78

CHAPTER FIVE – Inundative Inoculations of the Tobacco Beetle, *Lasioderma serricorne* with Water Formulations of the Entomopathogenic Fungus, *Beauveria bassiana*

5.1	Introduction	82
5.2	Materials and Methods	83
5.2.1	Maintenance and culture of experimental organisms	83
5.3	Preliminary pilot study	85
5.3.1	Inundative bioassays using cultures of two different ages	86
5.3.2	Scanning electron microscopy (SEM)	87
5.3.3	Data analysis	87
5.4	Results and Discussion	88
5.4.1	Preliminary pilot study	88
5.4.2	Inundative bioassays using cultures of two different ages	90
5.4.3	Scanning electron microscopy (SEM)	95

CHAPTER SIX – Summary and Conclusions

6.1	Sourcing, Maintenance and Viability Assessments of EPF	98
6.1.1	Radial growth at different temperatures	99
6.2	Insect-Pathogen Interactions	100
6.2.1	Summary on EPF against <i>Sitophilus zeamais</i>	100
6.2.2	Summary on EPF against <i>Lasioderma serricorne</i>	104
6.3	Conclusions	105

References	108
Appendix 1	119
Appendix 2	120
Appendix 3	126
Appendix 4	127
Appendix 5	129
Appendix 6	130

LIST OF FIGURES

Figure 1.1: A - shows an adult <i>Sitophilus zeamais</i> . B - shows larvae remove from within maize kernel. Larvae of this species lack true legs (Sallam, 1994).....	20
Figure 1.2: Adults (centre: A and B), pupae (far left: A), larvae (right: A and C) of <i>Lasioderma serricorne</i> : (Slide courtesy of ICI Americas, Inc. http://ipmworld.umn.edu/) (Clemson University - USDA Cooperative Extension Slide Series: Pest Control Portal Copyright © 1999 – 2003).....	21
Figure 1.3: Spore-bearing structures of (a) <i>Beauveria bassiana</i> , which produces cream-white conidia alternately on an extending tip of a conidiophore. (b) <i>Metarhizium anisopliae</i> , which produces green conidia in chains from phialides. [Courtesy of Jim Deacon, the University of Edinburgh © Jim Deacon] (http://helios.bto.ed.ac.uk/bto/FungalBiology/index.htm#top).....	29
Figure 1.4: Germination of a fungal hyphomycete <i>M. anisopliae</i> (Taken from Boucias and Pendland, 1998).....	30
Figure 1.5: Spore germination and penetration of fungal Hyphomycete (Taken from Boucias and Pendland, 1998).....	31
Figure 2.1, a: Map of South Africa highlighting North West Province.....	47
Figure 2.1, b: Map of North West Province, South Africa - highlighting towns around which soils were collected. (www.cyberprop.com/northwest/ Properties%20north).....	48
Figure 2.2: Heavily mycosed <i>Galleria</i> larvae cadaver exhibiting stroma. EPF isolate PPRI 04305 (MCBG1).....	49

Figure 2.3: *In vivo* maintenance of fungal cultures on Sabouraud dextrose agar plates using larvae of the greater wax moth *Galleria mellonella*. A and B show fungus covered cadavers, 48-72 hours after death due to exposure to dry *B. bassian* spores. C shows mycosed cadavers four weeks after death due to EPF infection (conidial mat spreading out of the mummified larvae on to media).....51

Figure 4.1: Innovative Lure dubbed the MNS Tower– for selection of live adult insects from sieved population. A - Assembled tower with sieved insects in 2, the containment zone. B - View from the top showing wandering individuals gathering in 1, the collection chamber.....61

Figure 4.2: Example of germinated conidia of local *B. bassiana* 24 hours post inoculation (spread plate) to SDA and incubation at 25°C for 24 hours (A- represents isolate PPRI 04305, and B; represents PPRI 04306). (Spores viewed at X40 magnification, light microscope).....63

Figure 4.3: Scanning electron micrographs of the maize weevil (*S. zeamais*). A - shows head of mycosed adult weevil infected with *B. bassiana*. B - shows head of uninfected adult weevil included for comparison with A to differentiate between fungal growth and insect physiology.....72

Figure 4.4: Scanning electron micrographs of the maize weevil (*S. zeamais*) A - shows mycosed cadaver of an adult weevil exhibiting fungal trough body segments. B - shows leg of maize weevil showing emergence of *B. bassiana* growth from join.....72

Figure 4.5: Top- left to right shows mycosed cadavers inoculated with A= 10^8 , B= 10^7 and C= 10^6 conidia ml⁻¹. Bottom- left to right shows cadavers inoculated with D= 10^5 and E= 10^4 conidia ml⁻¹. Photographs taken seven days after incubation to stimulate mycosis.....74

Figure 4.6: Fungal growth of isolate PPRI 04306 at different temperatures (top a=15, b=20 and c=25°C) (bottom d=30 and e=37°C) after a 4 day incubation period.....77

Figure 4.7: A - example of mycosed weevil cadaver used in dry spore experiment. B - Maize seed damage between untreated and fungus treated plots – top control plots with no EPF infected cadavers, bottom (left IMI 386 701 right PPRI 04307) after two months incubation at 25°C.....79

Figure 5.1: Innovative sampling device designed to attract adult pests into collection area. A - shows a view from above of the MNS Tower where cigarettes were added as an attractant for fit tobacco beetles. B - shows assembled MNS Tower.....84

Figure 5.2: Mycosed tobacco beetle cadavers resulting from inoculations with water formulated *B. bassiana*. A - isolate IMI 386 696; B - isolate PPRI 04305; C - isolate PPRI 04306 and D - isolate PPRI 04307.....89

Figure 5.3: Mycosed tobacco beetle cadavers resulting from; left (A, C and E) fresh inoculum infections and right (B, D and F) mature inoculum infections with 10^8 conidia ml^{-1} water formulations of local isolates (A and B - isolate PPRI 04307, C and D - isolate PPRI 04305 and E and F - isolate PPRI 04306).....93

Figure 5.4: Scanning electron micrographs of a mycosed adult tobacco beetle. A - shows whole body of mycosed cadaver. B - shows head and thorax of mycosed adult. C - shows close-up of fungal spores and mycelia emerging from highlighted area of B.....95

Figure 5.5: Scanning electron micrographs illustrating mycosis of adult tobacco beetle. A - shows head of a cadaver with sporadic fungal growth. B - shows mycelial and spore production of the fungus from the highlighted area of A. C - shows spores (red circles) and mycelia (blue circles) within fungal mass emerging from the cadaver.....96

Figure 5.6: Scanning electron micrographs illustrating fungal growth (mycosis) on the inner wing casing of an adult tobacco beetle resulting from the infection process of *B. bassiana*. A and B show mycelia and spores emerging from an adult tobacco beetle at different magnification.....97

LIST OF TABLES

Table 1.1: Major Events in the History of Microbial Pest Control.....	9
Table 1.2: Taxonomic Chart of Important Insect Pests of Stored Products.....	18
Table 1.3: Outline Classification of EPF and Examples of Insect Hosts.....	27
Table 2.1: Sourced Entomopathogenic Fungi.....	50
Table 3.1: Effect of Varying Temperatures on Radial Growth Rate (mm d ⁻¹) of Nine EPF Isolates Cultured on SDA Media.....	57
Table 4.1: <i>B. bassiana</i> Isolates Tested against Adults Maize Weevils.....	64
Table 4.2: Maize Weevil Mean Percentage Mortality and Mycosis from Inundative Water Formulation Inoculations of <i>B. bassiana</i>	69
Table 4.3: Effects of Various Conidial Concentrations on Fungal Virulence against the Maize Weevil.....	73
Table 4.4 Effect of Temperature on Mean Insect Mortality and Mycosis.....	76
Table 4.5: Maize Weevil Mortality from Infection by Dry Conidia.....	78
Table 5.1: Treatments Included in Experimental Bioassays.....	86
Table 5.2: Results from a Pilot Study Conducted to Determine Tobacco Beetle Susceptibility to <i>B. bassiana</i>	88

Table 5.3: Tobacco Beetle Mortality Data from Water Formulated Inoculum of Fresh Cultures of <i>B. bassiana</i>	90
--	----

Table 5.4: Tobacco Beetle Mortality Data from Water Formulated Inoculum of Mature (four month old) Cultures of <i>B. bassiana</i>	91
--	----

LIST OF ABBREVIATIONS

BCA - biological control agent

Bt - *Bacillus thuringiensis*

BV - baculoviruses

DSW - distilled sterile water

EPF - entomopathogenic fungi

GAP - good agricultural practices

NPV - nuclear polyhedrosis virus

OMA - oatmeal agar

PDA - potato dextrose agar

SDA - Sabouraud dextrose agar

SE - standard error

SEM - scanning electron microscopy