UNIVERSITY OF KWAZULU-NATAL

DEVELOPMENT OF FUNGAL BIOLOGICAL CONTROL OF FOUR AGRICULTURALLY IMPORTANT PESTS, *Sitophilus oryzae, Trialeurodes vaporariorum, Planococcus ficus* and *Eldana saccharina,* IN SOUTH AFRICA

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Development of fungal biological control of four agriculturally important pests, *Sitophilus oryzae, Trialeurodes vaporariorum, Planococcus ficus* and *Eldana saccharina,* in South Africa

by

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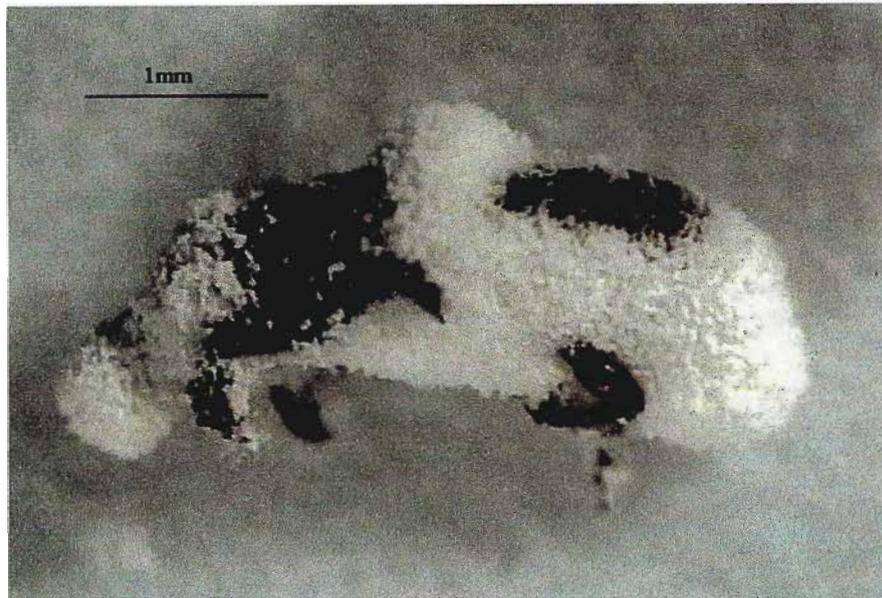*Faculty of Science and Agriculture*
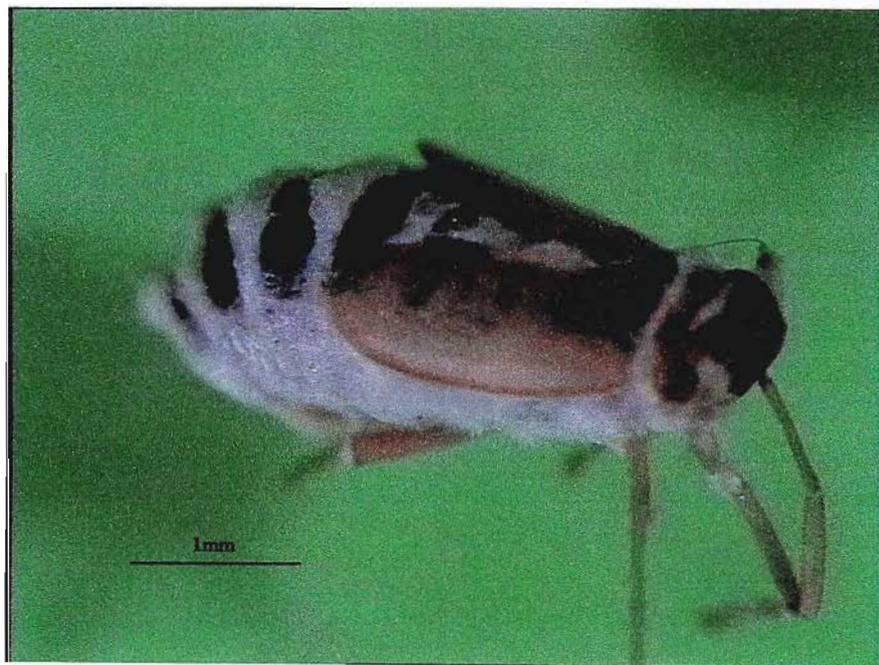*University of Kwazulu-Natal*
Pietermaritzburg
Republic of South Africa

August 2005

FRONTISPIECE -   FUNGAL BIOLOGICAL CONTROL

Rice Weevil, *Sitophilus oryzae*, infected with *Beauveria bassiana* (white mass)

External sporulation (white mass) of *Beauveria bassiana* on a leafhopper

ABSTRACT

The use of entomopathogenic fungi to control agriculturally important pests, both in greenhouses and in the field, has been demonstrated by various authors for a number of years. This has been brought about by the development of resistance in certain pest species to chemical applications and a growing public awareness of the safety implications of residual insecticides. Several entomopathogenic fungi were tested against four insect pests found in the Republic of South Africa (RSA), the greenhouse whitefly, *Trialeurodes vaporariorum*, the rice weevil, *Sitophilus oryzae*, the grapevine mealybug, *Planococcus ficus* and the sugarcane stem borer, *Eldana saccharina*. Further concentration, temperature and humidity studies were conducted with selected isolates on the rice weevil, *S. oryzae*.

Sitophilus oryzae is considered one of the most important pests of stored grain. Several fungal isolates were tested against the rice weevil, four of which, B1, PPRI 6690, PPRI 6864 and PPRI 7067, were selected for further testing based on the mortality results over a 21d period. Varying conidial concentrations were applied and at high doses of 1×10^6 conidia ml^{-1} with mortality rates of to 84% achieved. LT$_{50}$ values ranged from 6 - 68d. Increased spore concentration resulted in an increase in overall mortality.

Temperature and humidity was found to affect the infection potential of the four isolates tested. Four temperatures ranging from 15 - 30°C were tested. The highest mortality rates were obtained at 25°C where mortality ranged from 46 - 65% in 14d. Mortality rates decreased with decreasing temperature, and no mortality was recorded at 30°C. Temperature was found to significantly alter the LT$_{50}$ values, increasing the LT$_{50}$ with decreasing temperatures. Decreasing the humidity resulted in an increased LT$_{50}$ and a reduction in the overall mortality rates. The mortality of *S. oryzae* ranged according to the RH and isolate. Isolates B1 and PPRI 6690 resulted in the highest mortalities of 80 and 83% at 92.5% RH, with LT$_{50}$'s of 6.3d and 6.4d, respectively.

Several entomopathogenic fungi were tested against *T. vaporariorum*, *P. ficus* and *E. saccharina*, three key pests of South African crops. Nine fungal isolates were tested against the greenhouse whitefly, *T. vaporariorum*, with mortalities ranging from 26.7 -
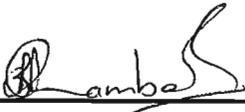
74.7% over 14d. *Beauveria bassiana* Isolates B1 and PPRI 6690 produced the highest mortality rates and were recommended for further pathogenicity testing against *T. vaporariorum*.

Planococcus ficus is a common pest of vineyards in the Western Cape Province, South Africa. Nine entomopathogenic fungi were screened against *P. ficus*, only two of which produced mortality. *Eldana saccharina* is a stalk borer, which infests sugarcane in large areas of Southern Africa. Five isolates were tested against second and third instar larvae, three of which, B1, PPRI 6864 and PPRI 6690 resulted in mortalities. Mean percentage mortality was low for all three isolates.

From the study it was evident that two of the isolates tested, B1 and PPRI 6690 (*B. bassiana*), showed potential against three of the four pests, and two isolates of *Lecanicillium lecanii* caused mortality in *P. ficus*. Further research and understanding of the effect of environmental conditions, spore concentration and epizootic potential would result in the further development of these isolates as future biological control agents.

DECLARATION

I, Craig Brian Chambers, declare that the research reported in this document was the result of my own investigation, under the supervision of Prof. M.D. Laing in the Discipline of Plant Pathology at the University of Kwazulu-Natal, Pietermaritzburg, from April 2002 to August 2005.

Craig Brian Chambers

August, 2005

Prof. Mark Laing

August, 2005

ACKNOWLEDGEMENTS

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CHAPTER 1

General introduction

1.1 Biological control

Biological control may be defined as the total or partial destruction of a pest or pathogen population by other organisms (Agrios, 1997).   The control of insects using biological techniques is becoming increasingly important as people become environmentally aware. Chemical control of insects has its associated problems, including the build up of resistance to a number of important chemicals, and population acceleration or resurgence. This result occurs when an organism has a positive reaction to a stress, which improves its ability to cope with environmental changes by means of new and better systems developed in answer to stress (Dittrich & Ernst, 1990). Initially in biological control, one or two control agents were used in conjunction with compatible chemicals. However, it is now common practice to use natural enemies and only resort to chemical control if necessary (van Schelt, 1993).

It has been known for a number of years that insect populations may be suppressed by certain fungi, either individually or total populations through epizootics. However, the success of fungal biocontrol agents depends on certain parameters as there are many variable conditions that result in an application being either unreliable or uneconomical. These parameters are specific to each biocontrol agent and would have to be established in the field to obtain successful control of the insect population. It is known that the impact of fungal biocontrol agents on their appropriate targets depends on their final formulation, the disposition of the insect host, and the physiological fitness of the material at the time of application (Kurstak & Tijssen, 1982). In order for a biological control agent to be successful it should:

    1) be able to spread amongst the pest population;

    2) be able to persist in the pest's environment;

3) be able to disperse through the environment;

4) be able to limit pest numbers to below that causing economic damage to plants following correct application;

5) be possible to produce or collect sufficient numbers of healthy viable specimens;

6) be specific and not destroy other biological control agents unless it is able to take their place adequately and

7) provide predictable control (Kurstak & Tijssen, 1982).

Many organisms have been used for biological control, ranging from protozoa to fungi, bacteria and even organisms of the same group as the pest. Research has shown that a number of fungal entomopathogens infect and control pest populations. A classical example of this would be the development and use of Green Muscle™ for the control of locust populations (Kooyman, 1999; Bateman, 2003). Green Muscle™ consists of spores of the fungus *Metarhizium flavoviridae* Gams & Rozsypal. Infected grasshoppers and locusts take approximately 6 - 10d to die. This fungus has proved to be a valuable means of locust control and has been registered in South Africa and many other African countries are likely to follow. Other microbes have proved effective and have been registered as control products, such as *Beauveria bassiana* and *Bacillus thuringiensis*.

1.2   Entomopathogens

Large numbers of entomopathogenic fungi are hyphomycetous, the majority being species in the genera *Beauveria*, *Metarhizium* and less commonly *Aspergillus*, *Hirsutella* and *Paecilomyces* (new name: *Isaria*) (Tanada & Kaya, 1993). Infection by this group of fungi occurs with the aid of conidia. There are four possible routes of entry into the host. The most common route of entry is directly through the outer integument. Other less common paths of infection are through the digestive tract, trachea and wounds on the insect (Cooke, 1977). Standardized practices were used for the isolation of fungal entomopathogens for testing in this thesis. These standard practices included using selective media (Veen & Ferron, 1966; Chase *et al.*, 1986;

Mitchell *et al.*, 1987; Lui *et al.*, 1993) and isolations directly from infected insects (Hatting *et al.*, 1999). Eight fungal cultures were obtained from the South African National Collection of Fungi at the ARC – Plant Protection Research Institute (ARC-PPRI) in Pretoria and were included in the study.

1.2.1   Host penetration by entomopathogenic fungi

Understanding the events that occur during the infection process of the host insect gives one an idea of where and how certain species of fungi attack and control insect populations. Entomogenous fungi can infect an insect at many stages during the insect's life cycle, i.e., egg, larval, pupal or adult. Fungi may live entirely superficially on the host's exterior or may penetrate the insect's integument, deriving their nutrients directly from certain internal parts of the host's body (Tanada & Kaya, 1993).

Fungi are considered to be entomopathogenic if the host becomes deformed in any way or its normal breeding habits are interrupted by the infection. It is thought that most fungi are saprophytic, feeding on dead organic matter, but under favourable conditions can colonize the exoskeleton. It has also been suggested that certain fungi may have evolved parasitic means of existence in order to escape interspecific competition (Agrios, 1997).

1.2.2   Fungal penetration of the host

Some insect pathogenic fungi enter their hosts through the epicuticle of the exoskeleton or cuticular layer. This layer contains various lipids, which have been reasonably well characterized (Anderson, 1979). It has been found that the alteration of this layer by light abrasion or washing with a non-polar solvent reduced the resistance of certain insects to fungal invasion (Wheeler & Blackwell, 1984). It has been reported that within exuviae, antifungal, medium chain saturated fatty acids, thought to be caprylic and capric acid, are found. It was thought that the reduction of resistance could be attributed to the removal of these compounds (Wheeler & Blackwell, 1984).

For spore germination to take place, favourable conditions are needed. For most fungal pathogens the humidity must be >80% and the temperature approximately 25°C for infection to occur (Wheeler & Blackwell, 1984; Vidal *et al.*, 2003). Once favourable conditions have prevailed, a germ tube arises from the conidium. Growth occurs over a short period of time before the tips of the germ tubes become enlarged, forming what is known as an appressorium (Figure 1.1). This structure, once matured, secretes a mucilaginous sheath, which anchors it to the host's cuticle (Tanada & Kaya, 1993). An effective appressorium then forms pressure points, which results in the distortion of the cuticular layer.
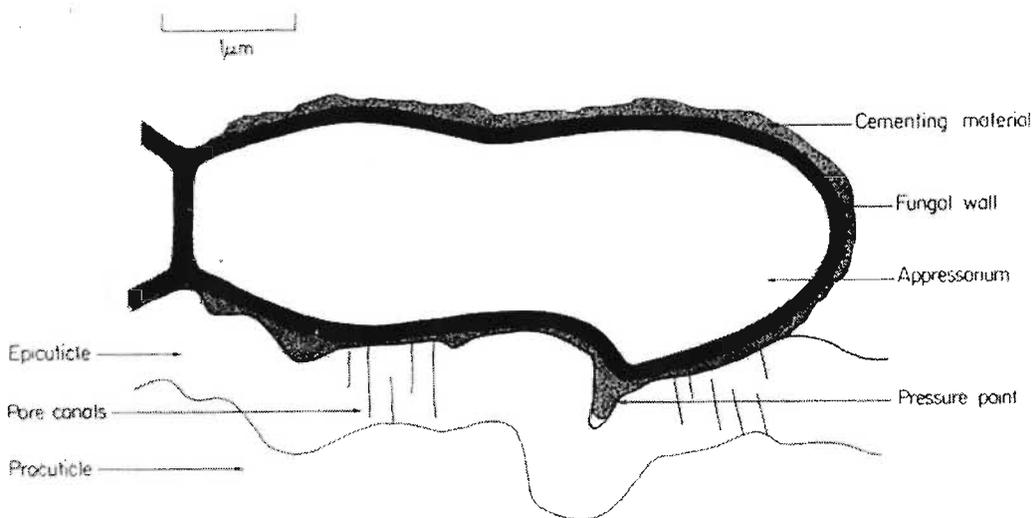
Figure 1.1   Appressorium arising from germ tube of conidia (Cooke, 1977).

Histolysis of the epicuticle occurs at the pressure points resulting in a cavity below each point. This induces the appressorial wall to degenerate and a fine penetration peg to grow into the newly formed cavity. The peg passes through the epicuticle into the procuticle and tip of the penetration peg expands (Figure 1.2). This results in the complete histolysis of the outer procuticle. From this plate, lateral and vertical

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hyphae are produced for increased penetration of the host's cuticle. The vertical penetration of the procuticle takes place in a stepwise manner (Cooke, 1977).
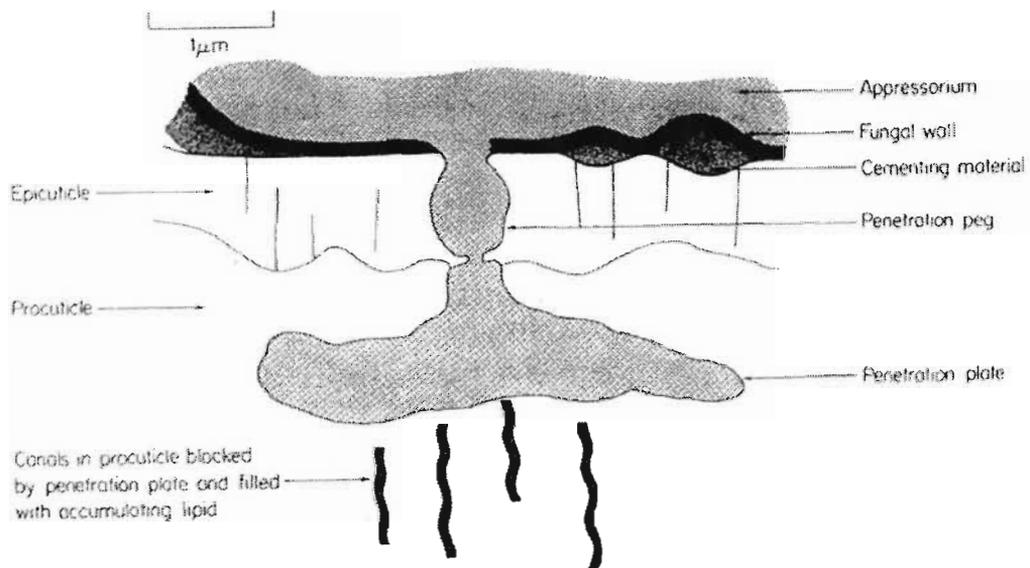
Figure 1.2   Formation of the penetration peg and penetration plate (Cooke, 1977).

Growth occurs vertically over a short distance before it gives rise to further chains or sheets of cells. Once the hyphae break free of the inner procuticle they enter the host's body cavity. It has been suggested that not only physical factors but also enzymes play a major role in fungal penetration of the exoskeleton of an insect (Wheeler & Blackwell, 1984).

The production of an appressorium does not always occur, even if the fungus has the ability to produce it. Invading mycelia are frequently narrower within the cuticle than in the internal cavity or haemocoel of the insect. They have the ability to penetrate between lamellae of the cuticle and cause infection (Fransen, 1990).

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1.2.3 Fungal-host interactions

Hyphae grow through or around cells, and thus the fungus infects the entire body of the host organism. Some fungi achieve colonisation of the host by producing yeast-like hyphal bodies. After colonisation of the body has occurred, certain processes follow. The hyphae, which were initially relatively short, lengthen and become longer as the disease progresses (Roberts & Yendol, 1981). The hyphae gain the ability to penetrate the host's firm tissues and internal organs. Fungal nutrition becomes necrotrophic because the fungus kills the host cell, then lives on it saprophytically. The death of the insect ends the parasitic development of the fungus (Tanada & Kaya, 1993).

Infection of the host by entomogenous hyphomycetes often occurs very rapidly with penetration occurring in less than 24hr and death of the host within 10d. Soon after infection, within 4 - 5d, symptoms of the fungal disease are often visible. For example, the host respiration may rise to a maximum about 1wk after infection (Cooke, 1977). Symptoms of infection may include loss of weight and hemolymph of the host becoming slightly acidic (Sussman, 1952).

After death of the host, the fungus spreads throughout the host's body. It forms a compact mass of mycelia within an intact integument. This allows the fungus to remain dormant within the protection formed by the integument of the host. When conditions are favourable, the hyphae emerge through the insect's integument. Conidiophores bearing conidia are produced, leading to sporulation.

1.3 Insect pests

Crop loss due to insect damage is a major concern in South Africa. Four well-known pests of South African agriculture were selected: the greenhouse whitefly, *Trialeurodes vaporariorum*; the rice weevil, *Sitophilus oryzae*, the grapevine mealybug, *Planococcus ficus* and the stem borer *Eldana saccharina*. Each of these

insects affects the attacked crop in different ways and will be addressed in further detail.

1.3.1   Greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood)

The major impact of whiteflies in agricultural cropping systems is the reduction in harvestable product and vectoring of important viruses. Whiteflies are well known vectors of plant viruses, which is the major concern when investigating their economic importance (Cohen, 1990). However, whiteflies are generally pests of disturbed systems, thriving on crops that were previously stressed. The search for effective chemical control has been ongoing for many years. Many chemicals have proven effective in the past, but the insect has the ability to develop high levels of resistance to most chemicals in a short period of time, rendering the chemical obsolete (Gindin & Ben-Ze'ev, 1994).

Although traditionally associated with tropical regions of the world, whiteflies are also found in the sub-temperate zones, such as South Africa. About 1200 whitefly species have been described in the Alerodidae (Scholtz & Holm, 1996). However, only a small percentage of these species are considered to be pests. One agriculturally important whitefly is the greenhouse whitefly, *T. vaporariorum* (Westwood) (Anneke & Moran, 1982). The adult whiteflies are small (a wing span of only about 3mm) and covered with a layer of white powdery wax with the exception of only a few species, hence the name 'whitefly'. Morphologically they resemble the family Psyllidae but are distinguished by having fewer antennal segments as well as a greatly reduced wing venation. The last abdominal segment of both the adults, as well as the nymphs, bears a large dorsal opening, known as the vasiform orifice, which receives the anus and is characteristic of the aleyrodids (Scholtz & Holm, 1996).

1.3.1.1 Whitefly biology

Whiteflies reproduce sexually and are oviparous. The eggs are laid on the host plant tissue. The nymphal instars are flattened and feed on the sap of the plant tissue. The life cycle includes and egg, four nymphal instars, a pupa and the adult. The final

instar moults to form the pupa, which then gives rise to the adult (Scholtz & Holm, 1996). Temperature and humidity affects the developmental time of each stage of the life cycle. The lower threshold of development is 8°C and the upper is 35°C. The lower and upper limits differ amongst developmental stages (van Lenteren & Noldus, 1990). Soto *et al.* (2000) investigated the effect of temperature on the development time of *T. vaporariorum* on tomato. They found that as the temperature was increased from 8 - 31°C the development from egg to adult was reduced from 130.3 to 18.4d. Fecundity is highly variable and is influenced by the species, cultivar and physiological stage of the host plant (van Lenteren & Noldus, 1990). The generalized life cycle of a whitefly is shown in Figure 1.3.
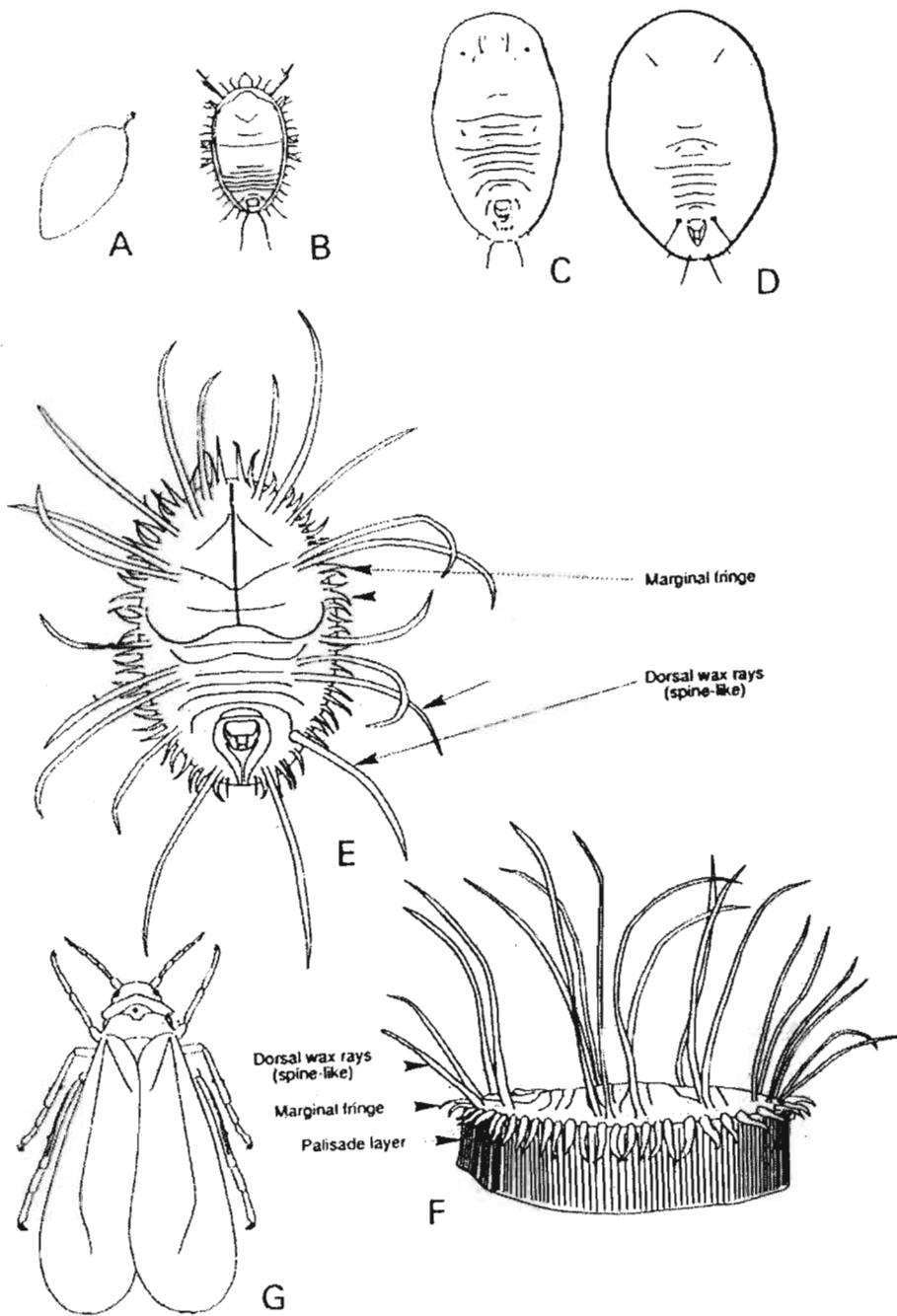
Figure 1.3. Generalized life cycle of a whitefly. A. egg. B. crawler. C. second instar. D. third instar. E pupa, dorsal view. F. pupa lateral view. G. adult whitefly (Gill, 1990).

1.3.1.2 Fungal biological control

There are five fungal genera that are well represented in the biological control of Aleyrodidae, namely *Beauveria, Cordyceps, Aschersonia, Paecilomyces,* and *Lecanicillium* (Fransen, 1990; Vidal *et al.*, 1997, 2003; Feng *et al.*, 2004).

The genus *Aschersonia* may one-day be important in the biological control of whiteflies due to this genera being specialized pathogens of whiteflies (Homoptera: Aleyrodidae) and scale insects (Homoptera: Coccidae) (Meekes, 2001) (Table 1.1). Meekes (2001) tested 44 isolates of *Aschersonia* against *Bemisia argentifolii* (Bellows & Perring) and *T. vaporariorum*. Infection levels varied from 2 - 70% for the isolates tested. It was also noted that *T. vaporariorum* had a higher mortality. Further studies indicated that LT_{50}'s of between 4.6 and 8.7d were achieved for *T. vaporariorum* whereas *B. argentifolii* had LT_{50}'s of 4.5 – 9.9d.

Even though these fungi are specific and are known to be potential biological control agents, little is known, and more research is needed to elucidate the specific characteristics of these fungi.

Table 1.1   *Aschersonia* spp. and respective aleyrodid hosts (Fransen, 1990).

	Aschersonia sp.	Host Insect (Hemiptera: Aleyrodidae)
1	*A. aleyrodis* Webber	*Aleurothrixus floccosus* (Maskell)
		Trialeurodes floidensis (Quaintance)
		Bemisia giffardi (Kotinsky)
		Trialeurodes abutilonea (Haldeman)
		Tetraleurodes acaciae (Quaintance)
2	*A. andropogonis* Hennings	*B. giffardi*
3	*A. aurantiaca* Petch	*Paraleyrodes perseae* (Quaintance)
4	*A. confluens* Hennings	*Trialeurodes vaporariorum* (Westwood)
5	*A. flava* Petch	*T. vaporariorum*
6	*A. goldiana* Saccardo & Ellis	*Dialeurodes citri* (Ashmead)
		T. vaporariorum
7	*A. hypocreoidea* Cooke & Massee	*Acaudaleyrodes Africana* (Dozier)
8	*A. papillata* Petch	*T. vaporariorum*
9	*A. placenta* Berkley &Broome	*T. vaporariorum*
		D. citri
10	*A. tamurai* Hennings	*T. vaporariorum*
11	*Aschersonia sp.*	*Aleurocanthus spiniferus* (Quaintance)

Of more interest are the broad-spectrum entomopathogenic fungi, i.e., *Beauveria bassiana*, *Paecilomyces* sp. and *Lecanicillium lecanii* (Zimm.) Zare & Gams (formerly known as *Verticillium lecanii*) (Table 1.2). They are able to infect insects belonging to different orders and therefore are not as species specific as other biocontrol agents. The pathogenicity of the different species of these fungi may differ in relation to different hosts.

Table 1.2   Taxa of entomopathogenic fungi other than *Aschersonia* spp. that affect whitefly (Fransen, 1990).

PHYCOMYCETES	DEUTEROMYCETES
Coelomomyces	*Aspergillus*
Myiophagus	*Beauvaria*
Entomophthora	*Hirsutella*
Massospora	*Hymenostilbe*
	Metarhizium
ASCOMYCETES	*Microcera*
Cordyceps	*Paecilomyces*
Hypocrella	*Spicaria*
Myriangium	*Lecanicillium*
BASIDIOMYCETES	
Septobasidium	
Uredinella	

The biological control of aleyrodids has been well published in recent years. Chandler & Heale (1990) conducted an experiment which investigated two strains of *L. lecanii*, Strain 1.72 ('Vertalec') used in the control of aphids as well as whiteflies and Strain 19.79 ('Mycotal') used in the control of *T. vaporariorum*. They investigated the growth rate at certain temperatures as well as the time it took for sufficient germination to take place. Strain 19.79 was found to germinate slower than Strain 1.72, but grew more rapidly once germination had taken place. Infection of these insects by entomopathogenic fungi is initiated by the germination of conidia on the host surface and is then followed by fungal penetration of the cuticle. Rapid germination of spores was correlated to pathogenicity of *L. lecanii*. The infection of the host cuticle by *L. lecanii,* relies on humidity. It has been found that, within a glasshouse a period of high humidity, coinciding with the application of *L. lecanii* conidia, should be of sufficient time (8 - 10 hours) for only a small portion of the

conidia to germinate on the host. Therefore, the patterns of humidity change within a glasshouse or in the field have a major effect on the infection of the host and thus the pathogen's pathogenicity (Chandler & Heale, 1990). Further research by Chandler *et al.* (1993) investigated the effect of mixing two strains of *L. lecanii* and their potential as a biological control agent of *T. vaporariorum* and the aphid *Macrosiphoniella sanborni* (Gillette). The mean pathogenicity of the mixture of the two strains was intermediate to that of each individual strain. This was attributed to the competitive interaction between the two strains during infection. When the host was infected with the dual-strain suspension, only mycelium from Strain 19.79 was recovered from *T. vaporariorum*. Competition between fungal strains on the same host occurs as a result of the differential expression of characteristics important to pathogenicity. The final result of the experiment suggested that, under glasshouse conditions, simultaneous use of two strains of *L. lecanii* should have little or no advantage over single strain efficiency. Long-term differential sporulation of the strains on the host may lead to the exclusion of one of the strains. Therefore, repeated application would eradicate this problem and thus would stabilize the strain balance (Chandler *et al.*, 1993).

Meade & Byrne (1991) considered using *L. lecanii* as a biological control agent for *B. tabaci*, the sweet potato whitefly. They investigated the effect of adding a surfactant, Triton X-100 to the entomopathogen. Results indicated that the whitefly eggs had an extremely low rate of infection, but the proportion of nymphal mortality due to fungal infection was significantly higher than those receiving other treatments of only surfactant. It was assumed that the surfactant adversely affected *B. tabaci*, resulting in an increased infection. The outer cuticle or exoskeleton of the insect presents the main barrier to the entomopathogen. A chemical that weakens this barrier, and has no adverse affects on non-target species, could be used in conjunction with an entomopathogen to increase fungal infection.

Vidal *et al.* (1997) investigated the pathogenicity of 30 isolates of *Paecilomyces fumosoroseus* (Wize) Brown & Smith against *Bemisia argentifolii*. The results varied

greatly with only a single strain producing total mortality. The fungus resulted in mortality of all the instars. Although high virulence is one of the prerequisites of a potential biological control agent, the results obtained by the experiment under optimal laboratory conditions do not guarantee that the fungus will be a successful biological agent in the field. The laboratory results need to be tested under simulated and actual target environments. The laboratory results were promising and did confirm the potential for the fungus *P. fumosoroseus* to be used as a microbial control agent of specific whitefly populations.

It is important to establish if the use of a fungal biocontrol agent influences the natural predators of the target insect in any way. The loss of natural predators may have an ecological impact on the environment. This could even result in an increase in pest populations if the introduced biological control agent is not as effective as the original natural predators that were present. Entomopathogenic fungi and insect natural enemies have the potential to complement or interfere with one another, depending on the environmental conditions (Lacey *et al.*, 1977). Poprawski *et al.*, (1998) studied the lethal and sub-lethal effects of the entomogenous fungi *B. bassiana* and *P. fumosoroseus* against *Serangium parcesetosum*, a coccinellid predator of whiteflies. The results varied but feeding on whitefly larvae infected with *B. bassiana* caused an 86% mortality of the coccinellid immatures whereas there was little effect on the coccinellid immatures when ingesting whiteflies infected with *P. fumosoroseus*. Further knowledge is needed to adjust the timing of various releases of biological control agents to the maximum additive effectiveness in the field without impacting the natural predators to any great extent.

In a trial where *Paecilomyces fumosoroseus* and *B. bassiana* were applied against *Bemisia tabaci* (Wraight *et al.*, 2000), control levels of 86 to 98% were achieved with both pathogens following repeated applications of varying conidial rates at 4 - 7d intervals. There have been a number of recent publications reporting significant population suppression by various applications of entomopathogenic fungi on various crops (Wraight *et al.*, 1998; Liu *et al.*, 1999).

1.3.2 Rice weevil, *Sitophilus oryzae* (L.)

The rice weevil belongs to the beetle family Curculionidae, the largest insect family, containing more that 45000 described species. Southern Africa supports approximately 25% of the sub-Saharan curculionid fauna (Scholtz & Holm, 1996). The rice weevil is considered one of the most serious stored grain pests worldwide (Batta, 2004). It originated in India and has been spread worldwide by commerce and now has a cosmopolitan distribution. Both the adults and larvae feed on whole grains. They have a wide host range, feeding on wheat, maize, oats, rye, barley, sorghum, buckwheat, dried beans, cashew nuts, wild birdseed, and cereal products (Arbogast, 1991).

The adults are small, approximately 3 - 5mm in length and stout in appearance. It is very similar in appearance to the granary weevil (Scholtz & Holm, 1996). However, the rice weevil is reddish-brown to black in colour with four light yellow or reddish spots on the corners of the elytra (Figure 1.4). The pronotum is almost equal to the length on the abdomen. The head with snout is as long as the prothorax or the elytra. The prothorax is strongly pitted and the elytra have rows of pits within longitudinal grooves. The larvae are legless and stay inside the hollowed grain kernels. They are approximately 2 - 3mm in size with a cream coloured body and dark head capsule. Pupation occurs within the seed kernel (Scholtz & Holm, 1996).

Figure 1.4   *Sitophilus oryzae* (Website 1).

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1.3.2.1 Rice weevil biology

The adult female rice weevil lays an average of 4 eggs per day and may live for 4 - 5mo. The full life cycle may take only 26 - 32d during the summer months, but requires a much longer period during winter. At 27°C and 69% relative humidity the rice weevil completes its life cycle, from oviposition to adult emergence, in 35d (Arbogast, 1991). The eggs hatch in about 3 - 5d. The larvae feed inside the grain kernel for an average of 20d. The pupae are naked and the pupal stage lasts an average of 5d. The new adult will remain in the seed for 3 - 4d while it hardens and matures (Arbogast, 1991).

1.3.2.2 Control

The most important aspect of control is location of the source of the infestation, which may include stored seed, wild bird seed, dry plant arrangements that contain wheat or other seed heads, popcorn, beanbags and many stored grain products. Infested materials should be destroyed or disposed of. All life stages can be controlled by extreme temperatures. Egg hatching and insect metamorphosis can be completely inhibited by lowering temperatures in the grain storage areas below 10°C (Nakakita & Ikenaga, 1997). The best control measure is to store products likely to be infested in pest-proof containers of plastic, glass, or metal. Seeds and nuts can be stored long term by adding a 2.5cm cube of dry ice (solid carbon dioxide) to a 1l Mason jar of seeds and sealing the lid. Carbon dioxide is an alternative measure for controlling *S. oryzae* in stored grain. It is as effective as chemical control but does not have the contaminant effect (Moreno *et al.*, 2002).

Infestations in non-food areas can be treated with space sprays or crack and crevice treatments with residual insecticides registered against rice weevils. Infestations in large quantities of grain can be controlled by fumigation. However, there are associated problems with the use of chemicals on consumable goods. Most chemicals have a residual effect on the crop and therefore the grain has to be left for a period of time before it is suitable for human consumption. Therefore, other methods of control are being researched.

The use of fungal biological control agents such as *B. bassiana* has been addressed previously. Rice & Cogburn (1999) tested the activity of various *B. bassiana* strains against three coleopteran pests of stored grain. Their results showed an 80 - 100% adult mortality at high dosage levels (5 x 10^6 conidia/g of rice) for all three insects tested after a period of 21d. They also found a reduction in adult progeny of approximately 83 - 99% when using dosage levels of 5 x 10^6 and 2 x 10^8 conidia per gram of rice. Sheeba *et al.* (2001) found that the F_1 adult emergence was reduced by 86.2% after an application of 7.6log conidia/ml of *B. bassiana* to *S. oryzae*. The study confirmed that there is an increase in host mortality at higher doses of conidial suspension and this agreed with work done by Adane *et al.* (1996) and Rice & Cogburn (1999).

Moino *et al.* (1998) screened 72 entomopathogenic isolates, which included *B. bassiana* and *M. anisopliae* against *S. oryzae*, *S. zeamais* and *Rhyzopertha dominica*. The doses applied in the screening trial were high as insects were inoculated by walking over actively sporulating fungal cultures. Therefore even marginally virulent isolates would have resulted in some host mortality. However, *B. bassiana* produced the highest mortalities against the hosts and 10 isolates were selected for further experimentation. The initial screening trial showed that only 21.2% of the *B. bassiana* isolates screened produced more than 80% mortality of the host insect and no isolate of *M. anisopliae* resulted in mortality greater than 60%. Further testing of selected isolates on *S. oryzae* produced mortalities varying from 50 - 90%. LT_{50}s were calculated and ranged from 5.5 - 8.8 d for the different isolates.

1.3.3   Grapevine mealybug, *Planococcus ficus* (Signoid)

Mealybugs comprise the second largest bug family (Pseudococcidae) of scale insects in the world, with approximately 110 species in 50 genera are recognised in southern Africa (Scholtz & Holm, 1996). *Pseudococcus maritimus* Ehrh. and *P. ficus* are the two common species of mealybugs found on vines in South Africa. *P. maritimus* is characterized by having a uniform layer of white wax on its back. The waxy appendages are long; the last two being characteristically longer (Figure 1.5a). They

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are usually of a uniform thickness. Removal of the waxy layer on the body surface will result in yellow-red to light brown body colour. This species excretes only small amounts of honeydew (Scholtz & Holm, 1996). *Planococcus ficus* also has the waxy covering but it varies in thickness and one can usually see a light stripe down the middle of the back. The waxy appendages are short and are broader at the base than at the top (Figure 1.5b). Removal of the waxy covering will result in a dirty yellow to pinkish body colour (Picker *et al.*, 2002). This species characteristically produces large quantities of honeydew. Both these species have a broad host range and may be found on a number of different hosts. Joubert (1943) suggested that both species of mealybugs have a host range in South Africa of more than 80 different species of plant. Some of these hosts include ornamentals, pumpkins, vines, citrus, potatoes and various orchards.

The infestation of mealybugs on vines results in a poor appearance of the fruit and therefore affects the market value of the crop. For this reason mealybugs are a major pest of table grapes in RSA (Walton & Pringle, 2004a). An infected grape bunch will be covered in honeydew, produced by the insects, and results in the growth of sooty mould on the fruit, further reducing the market value of the crop. In the case of severe infestations, the vine may shed its leaves and the bunches of grapes generally shrink, giving the fruit a wilted appearance. Premature defoliation of a vine results in poor growth of the vine and thus poor crops (Annecke & Moran, 1982; Walton & Pringle, 2004b). Control of the pest can amount to more than R 1000 ha^{-1} season^{-1} for many of the 100 000 ha vineyards in South Africa (Nel *et al.*, 1999).
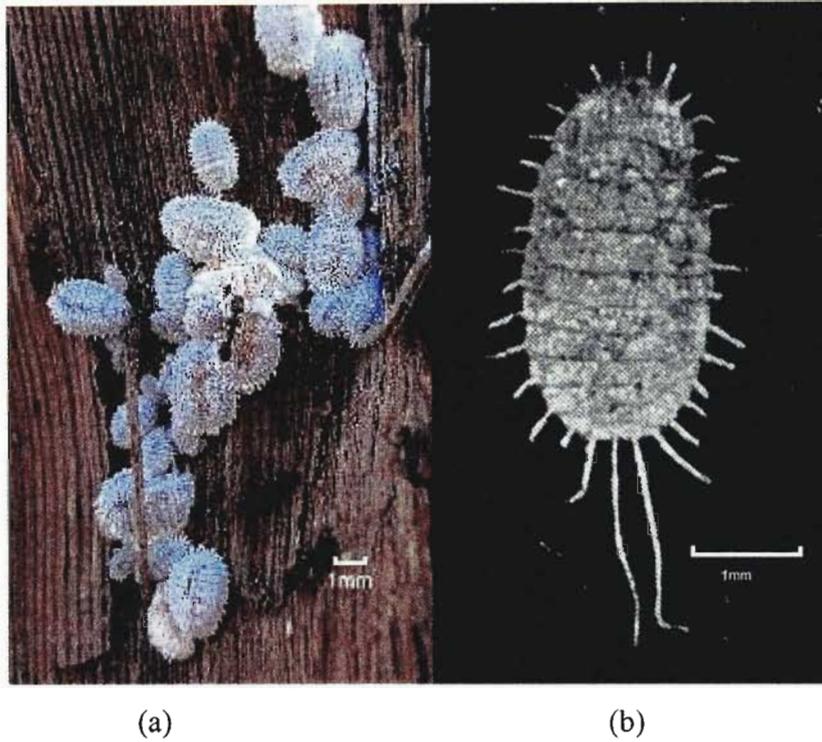
Figure 1.5 (a) – The grapevine mealybug *Planococcus ficus* (Website 2),

(b) – Adult female of *Pseudococcus maritimus* (Ehrh.)(Joubert, 1943).

Mealybugs have been a pest on vines since the mid-1930's. There has been much confusion as to the correct identification (Joubert, 1943). Only in 1975 was it shown that *Pseudococcus citri* Risso, thought to be the main mealybug pest on vines, was in fact rare and that the pest species on vines was *Planococcus ficus*. This species is said to have a global distribution. Vine mealybugs exploit their host very successfully (Annecke & Moran, 1982). During the winter months the mealybugs survive in sheltered spots beneath the bark of the grapevine. During the spring and summer months the mealybugs move from their place of shelter and infect the leaves, young shoots and fruit (Walton & Pringle, 2004b).

1.3.3.1 Mealybug biology

The female mealybug lays eggs in a protective ovisac of thin waxy strands. These ovisacs vary in size and also the number of eggs in each. The number of eggs per ovisac can vary from 100-500 (Joubert, 1943). The female generally lays eggs in a secluded area, such as under loose bark, folds in dry leaves or a contact point between bunches of the fruit. The female dies after the oviposition of the eggs.

The eggs are ovoid in shape and hatch after approximately 1wk in the summer but may take up to 2 - 3 mo during winter (Scholtz & Holm, 1996). A nymph crawler emerges from the egg usually moving from the emergence position to find a suitable area and begins feeding by inserting its long, thin mouthparts into the plant. The female nymphs undergo three moults. The life history of the male nymph is similar to that of the female up to the second moult. Prior to the second moult the male forms a cocoon in which it moults three more times. The male undergoes a pupal stage and finally emerges as a winged insect. Males have no mouthparts and thus only live long enough to reproduce. The life cycle of the pest may be completed in as little as 30d resulting in as many as 6 generations per year and a population of mixed ages and nymphal stages (Smit, 1964).

Mealybugs have very limited movement and therefore are unable to spread rapidly. Humans are the major means of the insect pest spreading. Both humans and animals that come into contact with heavily infested vines or fruit, inadvertently collect the pest in various stages of development and transport it around. Birds and insects may also be important vectors of the pest. The crawler stages move onto the bird or insect while it is feeding and are thus dispersed in this manner. Wind is also an important means of dispersal. The immature mealybugs are easily blown and carried by the wind currents to new and uninfected host plants (Joubert, 1943; Walton & Pringle, 2004b).

1.3.3.2 Control

Mealybugs are a problem in grape production and in many cases require chemical control to maintain the pest population below the economic threshold for the crop. This has been achieved by the application of various pesticides being applied to the crop at strategic stages in the lifecycle of the mealybugs. The use and timing of insecticidal sprays has been researched and spray programs are being used effectively (Joubert, 1943; Walton 2003). However, the use of insecticides has an adverse affect on the natural enemies of the mealybugs. These parasitoids and predators are considered very important in an integrated control approach. In many instances having sufficient natural enemies results in effective control of the pest population. The farmer is then able to limit the amount of insecticides used on the vines, which lowers production costs and increases profits.

However, the natural enemy complex may be interfered with both by man, using chemical applications, and by other insects, namely ants. Ants feed off the honeydew excreted by the mealybugs and in return protect the pest from insect parasitism and predation. This results in the natural enemies controlling fewer of the mealybugs and results in the population increase above the economic threshold of the crop. By controlling the ants in a vineyard it has been shown that the mealybug populations rapidly decrease in severity. It is recommended that ant control be the first requirement for the successful control of mealybugs on vines in South Africa (Addison, 2002; Joubert, 1943). Encouraging natural enemies in a vineyard can result in successful control of the pest population. There are a number of parasitic and predaceous insects, which are important natural enemies of mealybugs:

1. Hymenoptera, namely the families Encyrtidae and the Aphelinidae;
2. Coleoptera, members of the Coccinellidae;
3. Neuroptera, namely the families Hemerobiidae and the Chrysopidae;
4. Diptera, members of the families Cecidomyiidae and Chamaemyiidae (Walton & Pringle, 2004a).

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1.3.3.3 Mealybug discussion

Having a successful ant control program in place and encouraging the presence of natural enemies in a vineyard may result in sufficient control of a mealybug population. However more research is required as to the best and most effective methods in using beneficial insects as biological control agents.

Chemical control has been the most effective means of mealybug suppression in the past. Due to the problems associated with chemical applications and fumigations, i.e., destroying natural enemies, secondary pest outbreaks and resistance problems, other means of control are being researched. After an extensive literature search, there was no record of entomopathogenic fungal control of mealybug pests. This means of control therefore warrants further investigation. Control of mealybug populations with an effective fungal biological control agent has potential due to the life cycle of the pest. The pest spends part of its life cycle under the bark of the vines during the winter months. A spray application of fungal spores at this stage would result in favourable conditions for infection, due to the microclimate found under the bark of the vine. However, one needs to consider possible problems associated with spore application at this stage as the mealybugs are concealed. The spores need to come into direct contact with the pest in order for the infection process to begin. If these problems were to be solved, then this new and exciting approach to mealybug control may be realized.

1.3.4   The sugarcane borer, *Eldana saccharina* Walker

Eldana saccharina belongs to the Family Pyralidae of the insect Order Lepidoptera. This sugarcane borer is indigenous to Africa and is found to occur in numerous wetland sedges. It is known to be a pest of sugar cane, maize and other cereal crops in sub-Saharan Africa. Members of the Sub-family Gallerinae in South Africa, include other pests of economic importance such as the lesser wax moth, *Achroia grisella* Fabricius, the greater wax moth, *Galleria mellonella* Linnaeus and the rice moth, *Corcyra cephalonica* Stainton (La Croix, 1992).

E. saccharina adults are grey-brown in colour and have few distinguishing features other than two black dots near the outer margin of the forewing. The adults do not feed. However, they have been reported to drink water (Girling, 1978). Crop damage is caused be the larval stages of *Eldana*. The larvae bore into and feed within the stem of the host plant. It is difficult to accurately quantify crops losses due to *Eldana* damage, as estimates of these losses are not consistent (Conlong, 1994). Cane variety, growing conditions, and climate all affect the yield of cane crop. However, Smaill & Carnegie (1979) estimated the damage at 0.1% yield loss with every 1% of stalks damaged in South African cane.

1.3.4.1 *Eldana saccharina* biology

The life cycle of *E. saccharina* consists of four life stages, namely egg, larvae, pupae and adult. The female adult lays 100 – 200 eggs (Girling, 1978). The eggs are small oval structures and are usually yellow in colour at oviposition but change to orange as they develop. The duration of this stage depends on the climatic conditions. Atkinson (1980) reported that the developmental period of the egg varies with temperature. Eggs kept at a mean temperature of 17°C hatched after 12d and those kept at 26°C hatched after 5d. The eggs are generally laid in small batches (11-30) and in areas containing dead leaves, sugarcane stubble and dead shoots.

Newly hatched larvae are approximately 2 - 3mm long. These larvae are scavengers and tend to feed on decaying leaf matter. When they reach about 10mm in length they enter the stalk of the cane, either through cracks or by boring near nodes. *Eldana* larvae undergo between 5 – 8 larval instars depending on the quality of food available. The developmental time from larvae to pupae depends again on temperature, food and sex. The pupa is dark in colour and has a prominent dorsal ridge. The length of the pupal stage takes between 7 -14d (Girling, 1978). Equal numbers of male and female moths emerge, with the females generally being larger than the males. *E. saccharina* adults are grey-brown in colour and have few

brown in colour and have few distinguishing features other than two black dots near the outer margin of the forewing (Figure 1.6).
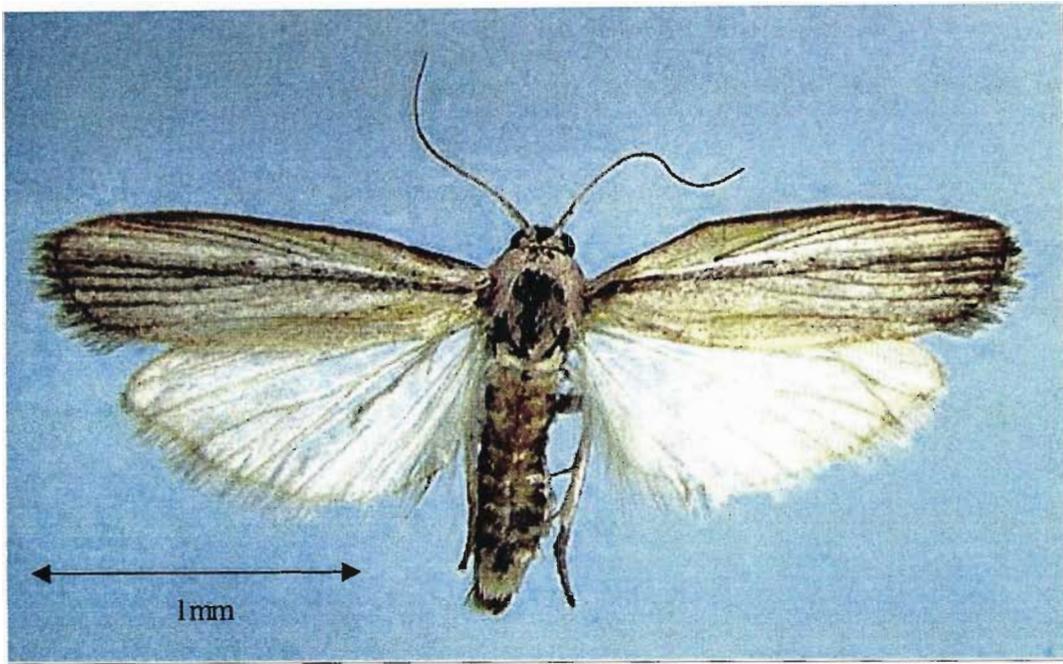
Figure 1.6   *Eldana saccharina* adult (Website 3)

1.3.4.2 Control

Various methods of control are used to limit pest population numbers. Chemical control of this pest has been widely used. However, timing of the insecticidal application is crucial due to the cryptic nature of the pest. Only the first to third instar larvae and the adults are outside of the stem and therefore can be targeted with spray applications. Studies into the use and timing of insecticide sprays have shown that chemical control of Eldana can produce good results (Leslie, 1997; 2000a; 2000b). The use of insecticides, although effective, can be detrimental to beneficial insects. Biological control of *Eldana* has been well researched and is being considered as an alternative method of control. Most of this research has involved classical biological control, as *Eldana* is indigenous to sub-Saharan Africa. A wide variety of natural enemies have been found to attack mostly *Eldana* larvae (Conlong, 1997; 2001). Two of the more common parasitoids include

Orgilus bifasciatus and *Goniozus indicus*. *O. bifasciatus* is the most common parasitoid of small larvae whereas *G. indicus* has been found to commonly parasitise the larger larvae. The only natural enemy common in all regions of Africa is the entomopathogenic fungus *Beauveria bassiana*. It has been well publicised that *B. bassiana* has been isolated from infected *Edlana* larvae during field sampling (Jacobs, 1989; Conlong, 1990; Mazodze & Conlong, 2003). However, little research has been conducted on the potential use of entomopathogenic fungi in the control of this pest. *Bacillus thuringiensis* has also been identified as a possible control option against *Eldana* (Jacobs, 1989, Downing *et al.*, 2000).

1.4   General problems with fungal biological control

Problems with biological control are real and need to be addressed. Application of pathogens as fungal biocontrol agents requires large quantities of the active agent. This usually means a higher cost due to the difficulties associated with mass production. Fungal pathogen production depends on whether or not the organism will grow *in vitro*. Otherwise the pathogen is produced in the living host or an alternative organism (Roberts & Yendol, 1981). This requires large numbers of the host and thus increases the cost of production and the product. Problems with formulation and the maintenance of fungal strains exist as well as limited shelf life of commercial products at room temperatures. Generally, there is a lack of adequate formulations for consistent pest control. Entomopathogenic fungi generally act slowly taking affect after 7d and in most instances frequent applications may be required in order to control multiple overlapping generations of the pest (Faria & Wraight, 2001).

The potential for fungal biological control depends largely on environmental conditions and the efficiency of the application method. Certain conditions favour the infection potential of certain fungi and if these are not met at the time of application, minimal control will be achieved. The cryptic habits of many insects make spore application very difficult, i.e., the preference of whiteflies to the abaxial surface of the leaf creates a difficult spray application problem when targeting these insects.

Pesticides are an appropriate control measure for pest organisms in many situations, whereas biological systems are usually only applicable to a few species or localized pest problems. However, there are exceptions such as *B. bassiana* and *Bacillus thuringiensis* Berliner, which infect a number of insects from different orders. This results in environmental problems such as the potential loss of species diversity.

1.5   General discussion

Biological control is becoming increasingly important as the public becomes environmentally aware and farmers are producing crops organically. Examples of biological control have been discussed throughout the article and have mainly come from a catalogue of successes. Alternatively, there have also been examples of experiments in biological control, which have failed and even had negative effects in some cases. This makes the point that biological control is not always guaranteed to succeed in every case. The success rate is likely to improve in line with our understanding of what constitutes a good biological control agent. To assess the overall value of biological control as a whole it is necessary to add the cost of unsuccessful introduction and negative environmental impacts to that of the successes.

Compared with chemical control, biological control is not necessarily cheap. Even when incorporating the unsuccessful attempts, the overall picture suggests that it may still be economically viable. Biological control is not easy to implement: detailed ecological and taxonomic understanding, trained manpower, continued monitoring and perseverance are all needed in order to establish a good or effective biological control agent. However, the rates of return are often favourable.

Probably the two best known agricultural and glasshouse pests in South Africa are whiteflies and aphids. Both these organisms cause great damage to crops and greenhouse plant alike. However, the main problem with them is that they are excellent vectors of viral   pathogens,   which   can   result   in   crop   damage.   Much   work

has been done on the biological control of whiteflies over the past ten years. Research has shown that *Aschersonia* sp., which are specific to the whiteflies, have a great potential for good biological control of whiteflies. Substantial work has been done on the broader-spectrum entomopathogenic fungi, *B. bassiana, Paecilomyces* sp. and *L. lecanii*. The pathogenicity of different stains of these fungi may differ in relation to different hosts, whereby making them partially selective. These entomopathogenic fungi have been found to produce varying results as to their effectiveness. However, positive results seem to outweigh the negative and therefore there is a potential for biological control of whiteflies within those three genera.

From the literature it is evident that there is potential for the fungal biological control of *S. oryzae*. Many positive results have been achieved with significant control of the test organisms in 5 - 8d. However, these trials are conducted under conditions favouring fungal infection of the insect host. In a stored grain environment the relative humidity and temperatures are usually far lower than that required for the entomopathogenic fungi to infect. Therefore, although one may have an effective pathogen, it may not be able to infect the pest population under the conditions required to store the grain. This is an interesting problem and requires further research into fungal formulations and application methods to maximise the infection potential of the fungal pathogen.

After an extensive literature search it was evident that no research has been done on the fungal biological control of mealybugs on vines. The mealybug behaviour of living under loose bark during the winter period provides a possible microclimate that may favour a fungal pathogen's infection process. Further research is required to substantiate this possibility.

The fundamental consideration in potential commercialisation of any of the entomopathogenic fungi is if an efficacious product can be developed. This product needs to be mass-produced cheaply enough to compete with the existing control strategies. Low-cost mass production of an entomopathogen is only one of a number of difficulties and technical constraints (Barlett & Jaronski, 1988). The biological

control of these insect pests has potential and could in time replace traditional chemical sprays. A future possibility would be an integrated control system in which natural predators, entomopathogenic fungal control and biodegradable insecticides are able to work together and supplement each other in the control of harmful agricultural pests.

The aim of the thesis was to test various entomopathogenic fungi against the selected target insect pests with further testing of the pathogens at different criterion, such as spore concentration, varying temperatures and relative humidities. The thesis follows the Dutch style of presentation.

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CHAPTER 2

Establishing effective techniques for rearing test insects in a glasshouse

Abstract

The greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), was reared using various methods. Rearing of these insects under laboratory conditions proved to be undesirable as the photoperiod could not be altered and the lighting provided was not of sufficient quality resulting in plant physiological problems. The use of large netted tanks and the glass rearing chambers did not result in sufficient numbers of clean test organisms. Rearing tanks were situated in a glasshouse cooled by a wet wall and fan system. The wet wall cooling system resulted in an elevated humidity and was unable to maintain temperatures below 30°C. The humidity levels reached over 90%, 11 hr d^{-1} in the greenhouse and 16 11 hr d^{-1} in the glass rearing tanks. This resulted in the insect cultures routinely becoming contaminated with entomopathogic fungi. These techniques for rearing whiteflies were discontinued after the 2001/2002 season. The wet wall was replaced with an industrial air-conditioning system in 2003/2004. The temperature was then maintained at 20°C (± 2°C) and the average humidity was lowered to 66%. These environmental conditions coupled with new ventilated rearing chambers produced viable whitefly cultures for screening of fungal entomopathogens. The methods used for the culturing of *Sitophilus oryzae* (L.) and *Planococcus ficus* (Signoid) produced sufficient numbers of clean test organisms so no new techniques were developed.

2.1   Introduction

Rearing insects is an essential part of fungal biological control research. Without healthy insect cultures one is unable to test the pathogenicity of fungal entomopathogens. Culturing of insects is time consuming and requires dedication on the behalf of the scientist (Forbes *et al.*, 1988). There are a number of different methods for culturing insects, each insect requiring its own specific environmental conditions and rearing techniques to obtain clean and pure test organisms.

Most aphids, particularly those of economic importance, are not difficult to rear in their parthenogenic phase. The principal requirements for the successful rearing of aphids include the following conditions:

1) a continuous supply of healthy plants of the correct species and physiological conditions;
2) an appropriate cage set up for the culture;
3) environmental conditions that inhibit the production of sexual morphs,
4) anchaintenance procedure which keeps the numbers of aphids in the culture under careful control (Blackman, 1988).

These conditions can also be applied to the rearing of whiteflies. The most important point with rearing insects is to produce an environment that favours the growth of the insect, but hinders the development of plant pathogens, insect pathogens and parasitoids. The environmental conditions that insects are subjected to may affect their morphology. This is also commonly seen when rearing aphids and similar effects are seen in whiteflies. Unfavorable conditions may result from overcrowding, poor plant physiology or temperature extremes (Dixon, 1985). The morphological size of whiteflies is affected when overcrowding occurs. Individuals under these conditions are smaller and less tolerant to environmental change (Gill, 1990).

The purpose of this study was to develop successful rearing techniques to produce sufficient numbers of healthy test insects in order to test the pathogenicity of various fungal entomopathogens.

2.2 Materials and methods

2.2.1 2001/2002 Season

2.2.1.1 Maintaining whitefly cultures

Initial rearings of *T. vaporariorum* (Westwood) were undertaken in a glasshouse at UKZN, Pietermaritzburg (29° 25' S, 30° 24' E). The temperature in the glasshouse was moderated by a wet wall and fan system (Figure 2.1). Various methods were used in order to obtain clean and pure whitefly cultures. The insect cultures were monitored for the development of plant and insect diseases as well as parasitoids and various spay applications were used to control the problems. The plants were spayed once a week with Bravo and Acrobat.
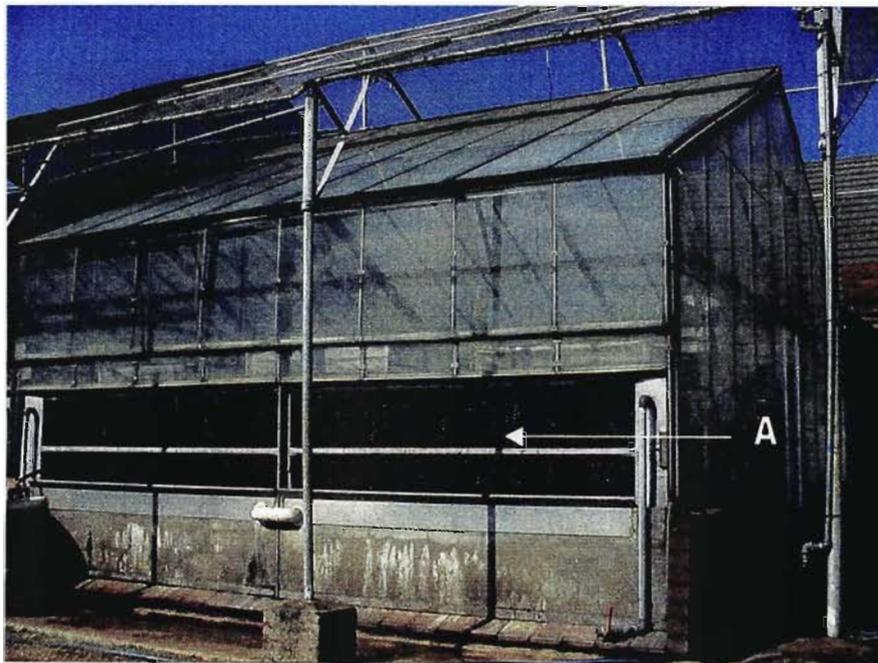
Figure 2.1   Glasshouse used for the rearing of *T. vaporariorum*. A - wet wall cooling system.

2.2.1.2 Laboratory culturing

The greenhouse whitefly was initially reared under laboratory conditions in an insectary maintained at UKZN, Pietermaritzburg, at the School of Botany and Zoology. The laboratory was kept at a constant temperature of 23°C (± 2°C) and was light with standard florescent globes with a 12/12hr light dark cycle. *Phaseolus* sp. (trade name: Gadra) were grown in 250ml Styrofoam cups with holes in the base, six of which were placed into a 2l plastic container. A standard potting medium was used in the cups. The bean plants were replaced as required. The plants were watered on a daily basis. Two 2l plastic containers with six plants in each, were placed into each rearing chamber. Each plant added to the chamber was inoculated with 15 adult whiteflies. The rearing chamber had dimensions of 80 X 50 X 50cm, and consisted of insect netting[1] along the longest two sides and the remainder of the tank was covered with clear plastic (Figure 2.2). The complete chamber had to be removed in order to add additional plants.
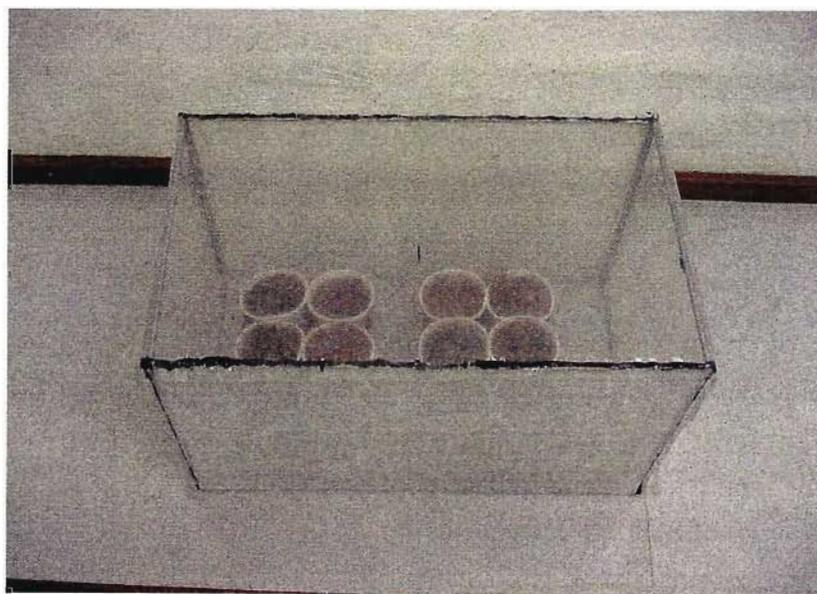
Figure 2.2   Insect rearing chamber used to rear aphids and whiteflies under laboratory conditions.

[1] Filmflex (Pty) Ltd, Durban, South Africa.

2.2.1.3 Insect netting tanks

Large insect growth chambers were made out of fine insect netting[1] and a steel framework. The tanks had dimensions of 200 x 120 x 120cm (Figure 2.3). Entrance to the tanks was obtained by removing the side nettings which were attached with Velcro to the framework. The plants were subjected to natural light conditions. Fifty potted *Phaseolus* sp. (Trade name: Gadra) were placed in each rearing chamber and each was inoculated with 15 whiteflies collected from the surrounding area. Standard potting medium was used to grow the bean plants. The bean plants were replaced as required. The plants were watered by a drip irrigation system, which was connected to an automated fertigation system. The plants were watered for one minute three times daily.
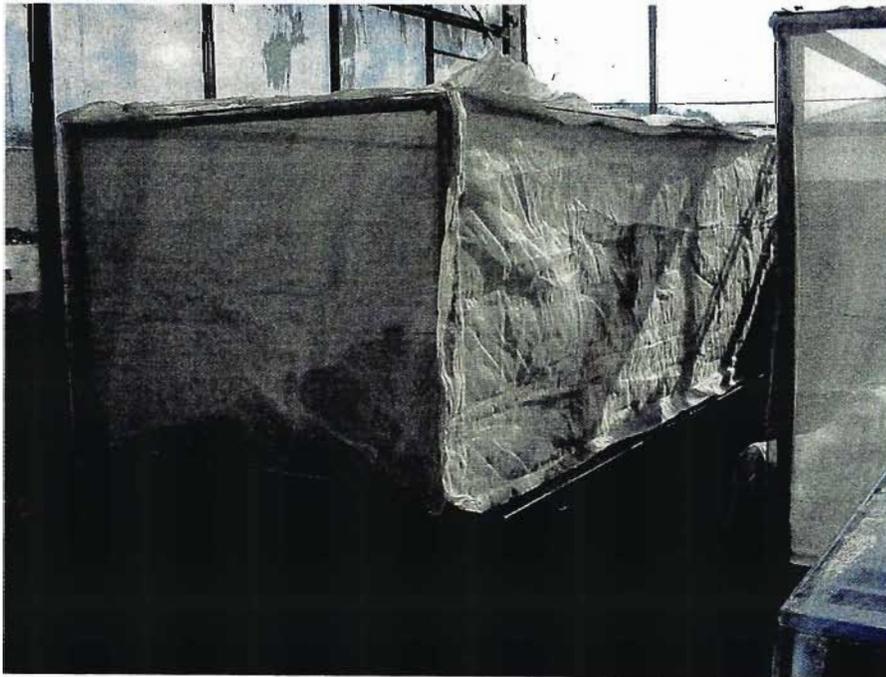
Figure 2.3   Netted insect rearing chambers used to culture whiteflies.

[1] Filmflex (Pty) Ltd, Durban, South Africa.

2.2.1.4 Glass rearing chambers

Glass tanks with dimensions of 120 x 45 x 45cm were used as rearing chambers (Figure 2.4). Wooden covers containing insect netting were used to seal the tanks and allow for ventilation. Twenty potted *Phaseolus* sp. (Trade name: Grada) plants were placed in each tank and inoculated with 40 whiteflies collected from the surrounding area. The whiteflies were collected with the use of an aspirator. The plants were watered on a regular basis by hand.

Figure 2.4   Glass tanks used as rearing chambers in the glasshouse under wet wall cooling.

2.2.1.5 Temperature and humidity

The temperature and humidity was monitored over a 9d period. To compare conditions within the rearing chambers and the greenhouse, a Hobo measuring temperature and humidity meter (manufacturer: Onset[1]) was placed in each respectively and set to take readings hourly for 9d. Weather conditions varied over the study period.

[1] Onset Computer, Mac Arthur Blvd, M.A. 02532

2.2.2 2002/2003 Season

The wet wall cooling system in the glasshouse was replaced late in 2002 by an industrial air-conditioning system and the wet wall was sealed with silage plastic to prevent airflow through the glasshouse (Figure 2.5). The air-conditioning system was set to maintain the temperature at $20^{\circ}C$ ($\pm 2^{\circ}C$).
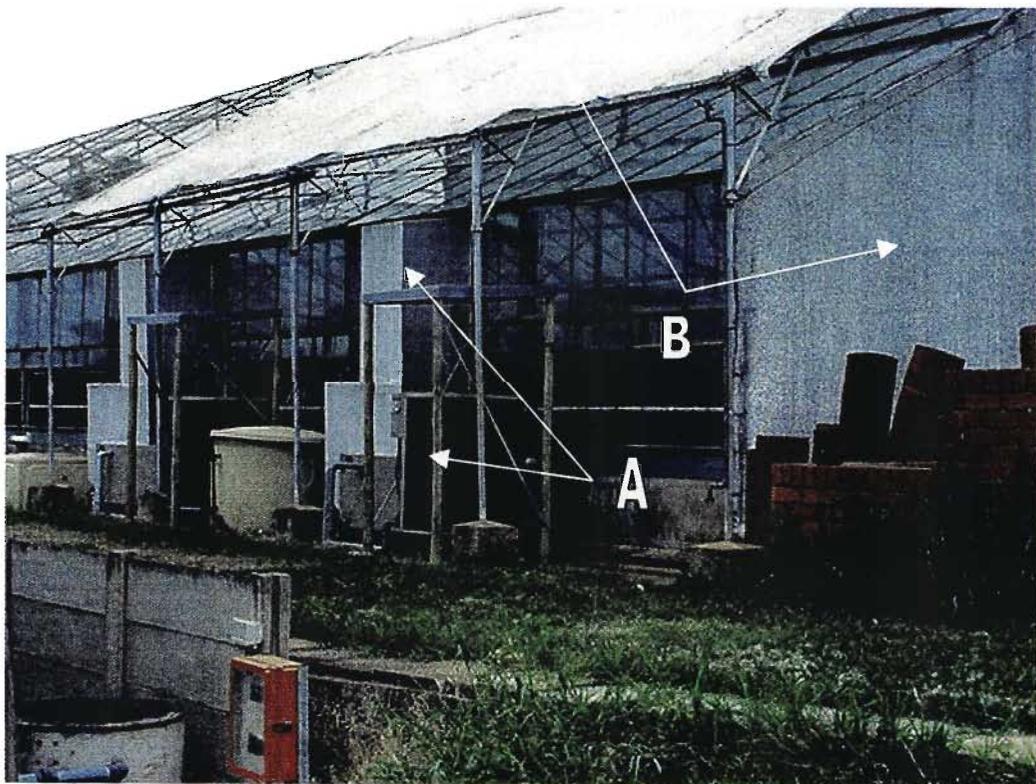
Figure 2.5   Air-conditioned glasshouse used for the rearing of whitefly colonies.

A- industrial air-conditioning unit,

B- shading to reduce the sunlight, lowering temperatures in the glasshouse.

2.2.2.1 Ventilated rearing chambers

The rearing chamber had dimensions of 150 x 50 x 50cm and consisted of fine insect netting[1] (sides and back) and Perspex (front and top). The bottom of the tank was a CR12 formed tray with drainage holes (Figure 2.6). The rearing tanks were watered by an automated fertigation system three times daily for one minute. The excess water was drained from the drainage points by hoses and directed into a drain to prevent excess water in the glasshouse in an attempt to lower humidity levels. Access to the whitefly culture was gained through the hinged Perspex lid of the cage. Ten plants were placed into each rearing chamber and inoculated with 40 whiteflies.

Figure 2.6   Ventilated rearing chambers. A: Perspex covering for the lid and front. The rest of the cage is covered with insect netting for ventilation.

[1] Filmflex (Pty) Ltd, Durban, South Africa.

2.2.2.2 Maintaining rice weevil cultures

Rice weevils, *Sitophilus oryzae* (L.), were obtained from an infested bag of sorghum seed. A rearing technique was developed to maintain rice weevil populations. The weevils were reared in 5l containers with ventilation holes cut into the lid and covered with insect netting. Clean sorghum seed was placed into the containers and inoculated by mixing with 200g of infested sorghum seed. New cultures were started every 6wk using the same method. The cultures were maintained in the air-conditioned glasshouse at $20^{o}C$ ($\pm 2^{o}C$).

2.2.2.3 Maintaining grapevine mealybug cultures

The mealybugs, *Planococcus ficus* (Sign.), were collected from infested vines and placed onto *Cucurbita maxima* (butternuts). The mealybugs were reared on butternuts, which were kept in plastic containers in a glasshouse at $20^{o}C$ ($\pm 2^{o}C$). The containers had dimensions of 40 x 25 x 15cm. The containers contained ventilation holes covered with standard mesh netting. New cultures were started by placing fresh butternuts on the lid of existing rearing. The butternuts were left for 3 - 4d for the first instar crawlers to move from the old cultures onto the fresh material. The butternuts were then removed and placed into separate rearing containers in the glasshouse.

2.2.2.4 Temperature and humidity

The temperature and humidity was compared between the glasshouse under air-conditioned temperature regulation and an identical glasshouse still under wet-wall cooling. The temperature and humidity was recorded with the use of a Hobo device. Each Hobo was placed in the center of each respective glasshouse and was covered by a Stevenson's screen.

2.3 Results

2.3.1 2001/2002 Season

The use of the laboratory method and the insect netting tanks proved to be undesirable for the culturing of clean pure whitefly cultures. The laboratory did not have lighting of sufficient quality to maintain healthy plants and therefore the rearing of sufficient numbers of test insects was not possible. The large insect netting tanks did not restrict the movement of insects resulting in mixed cultures of aphids and whiteflies. The use of these methods was therefore discontinued (Table 2.1).

Table 2.1   Assessment of the rearing techniques used for rearing *T. vaporariorum*.

	Rearing Technique	Insect growth	Plant disease growth	Entomopathogen presence	Parasitoid presence
1	Laboratory culturing	0	+	0	0
2	Insect netting tanks	++	+	+	+++
3	Glass rearing chambers	+++	+++	+++	++
4	Ventilated rearing chambers	+++	+	+	+

0	- no growth was evident
+	- growth of both insect and pathogens was slow and could not maintain sufficient numbers of whitefly
++	- growth was adequate
+++	- growth was exponential

Under wet wall conditions the average temperature inside the glasshouse was 19.9°C and inside the glass rearing chambers was 22.6°C. The maximum temperatures inside the glasshouse and the glass tanks were 32°C and 44°C, respectively. The humidity in both the glasshouse and rearing chambers reached 100% on a number of occasions during the study period. The average number of hours per day that the relative humidity rose above 90% was calculated for both the glasshouse and the

rearing chambers. On average the humidity rose above 90% for 11hr d^{-1} in the glasshouse and 16hr d^{-1} in the rearing chambers.

The use of the glass tanks as rearing chambers initially produced satisfactory results. The insect numbers grew rapidly and within 2 to 3wks substantial insect cultures were established. However, the cultures could not be maintained for any period of time due to a build up of insect pathogens, plant pathogens and parasitoids. The weekly use of Bravo and Acrobat had little effect on the plant pathogens. The whitefly cultures were regularly infected by fungal entomopathogens (Table 2.1).

2.3.2   2002/2003 Season

The removal of the wet wall cooling system and the installation of the air-conditioning system resulted in a lowering of the humidity and an accurate temperature control with fewer fluctuations. Figure 2.8 shows the comparative temperatures and relative humidities of the air-conditioned glasshouse and the wet wall glasshouse.
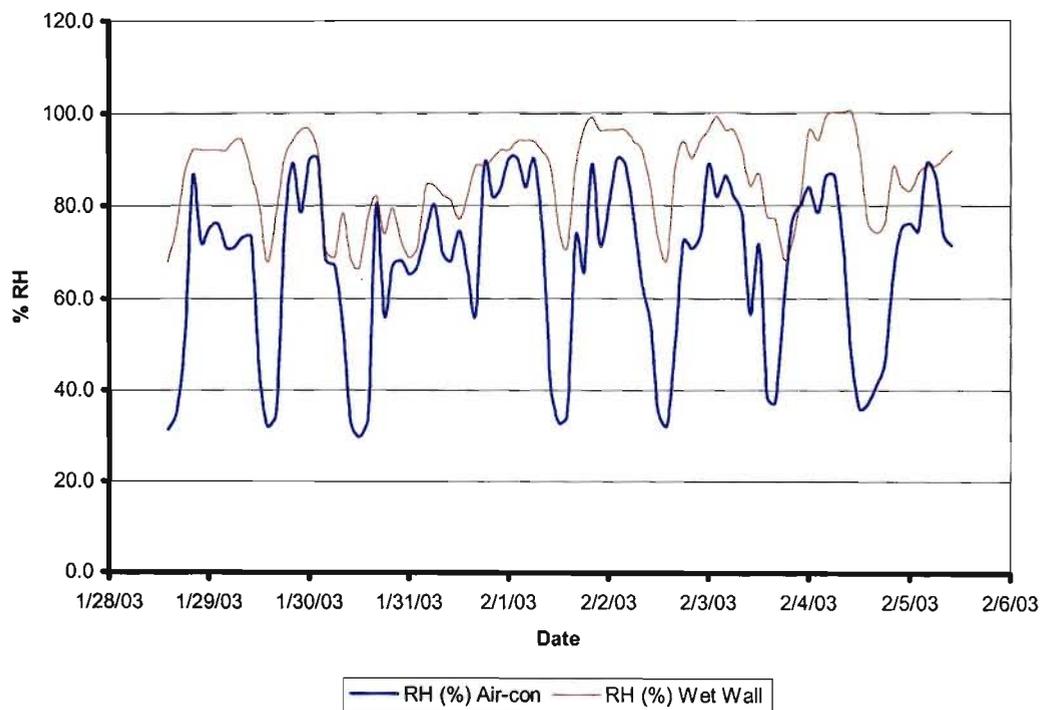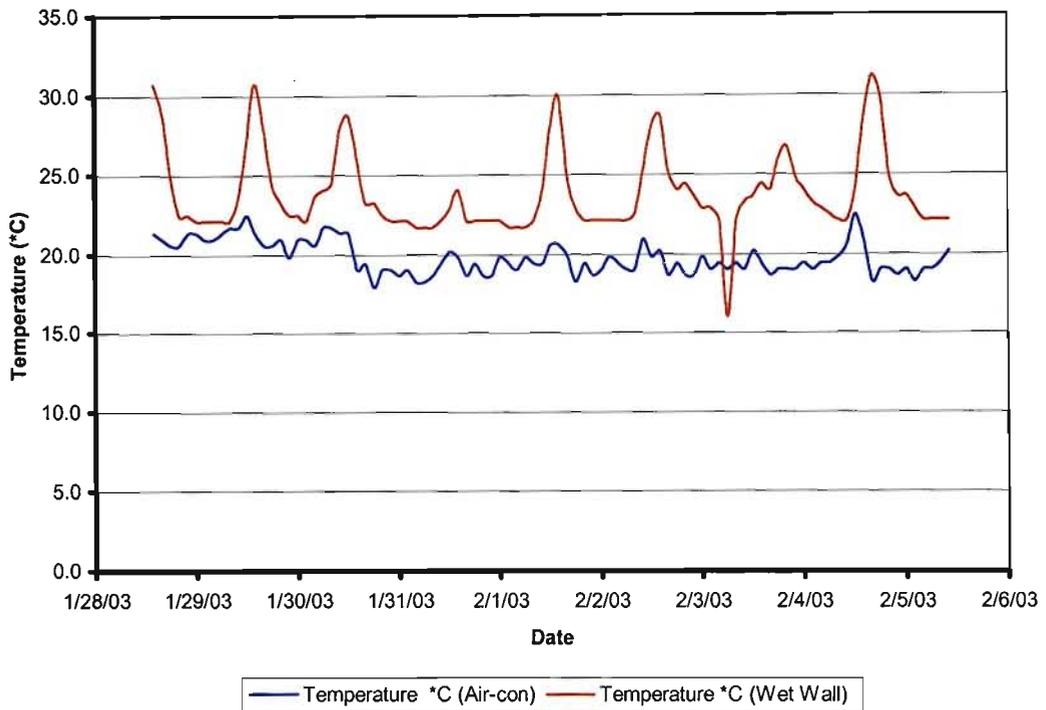
Figure 2.7   Temperature (top) and relative humidity (bottom) differences between the industrial air-conditioning system and the wet wall cooling system measured over a 9d period.

The average temperature over the 9d period in the air-conditioned glasshouse and wet wall cooled glasshouse was 19.9°C and 23.8°C, at the end of the 2002/2003 season. The average relative humidity was 66% and 86%, respectively.

The use of the ventilated rearing cages in the air-conditioned glasshouse produced healthy cultures of insects. Sufficient numbers of adult whiteflies were reared and the cultures remained relatively disease free throughout the study period. The rearing methods used for both *S. oryzae* and *P. ficus* resulted in sufficient numbers of healthy insects for screening purposes.

2.4 Discussion

Rearing sufficient and healthy insects in an important part of conducting research into fungal biological control. Many factors may influence the production of sufficient quantities of healthy insects. The use of the laboratory method proved to be undesirable for the rearing of whiteflies. The laboratory conditions were not conducive to plant growth. The plants did not have access to light of sufficient quality, as well as being subjected to 24 hours of fluorescent lighting therefore affecting the plant physiology. In order for a plant to remain healthy it has to undergo successful photosynthesis. Photosynthesis consists of two stages: a series of light-dependent reactions that are temperature independent and a series of temperature-dependent reactions that are light independent. These reactions need to take place cyclically over a time period for the plant to remain healthy (Salisbury & Ross, 1992). In the laboratory the plants were unable to carry out the dark reaction due to the continuous presence of light and therefore died within a number of days. The photoperiod could not be changed due to other experiments within the laboratory.

Culturing insects in the large insect netting tanks resulted in mixed insect cultures. One was unable to restrict the movement of other insect species into tanks. This was due to the size of the cages and the use of an automated fertigation drippers entering from below the cages. Insects could move freely onto the plants from the underside

of these tanks, resulting in a mixed cultures. The loss of insects due to parasitoids and entomopathogens was also evident. Using the standardized practices mentioned in Chapter 1, attempts were made to isolate and culture these entomopathogens. However, very few were isolated and those that were sporulated poorly in culture. The plants remained generally disease free in the netting tanks.

The whitefly population grew rapidly in the glass rearing tanks. However, the insect cultures and plants were frequently infected by both insect and plant pathogens, respectively, and therefore the cultures could not be used for further bioassays. For successful rearing of many different insects the environment in which they are cultured, should be controlled. In general the temperature should remain constant at approximately 20°C and the relative humidity should be kept between 40 and 60%. The insects in the glass rearing tanks were found to be subjected to temperatures ranging from 10°C - 43.9°C. The humidity in both the glasshouse and rearing chambers reached 100% on a number of days. This extended period of high humidity coupled with an average temperature of 19.9°C in the greenhouse and 22.6°C inside the glass rearing chambers resulted in optimum conditions for both fungal and insect pathogens (Appendix 2.1).

With the change from the wet wall cooling system to the air-conditioning cooling system the temperature and humidity was controlled at acceptable levels. This reduces the optimum conditions for insect and plant pathogen growth and resulted in healthier insect cultures. The ventilated rearing chamber had increased ventilation, which lowered the humidity in the insect culture and therefore resulted in little or no pathogen presence. This method for rearing whiteflies was continued thought the thesis and all the test insects were reared in this manner.

Sufficient numbers of healthy *S. oryzae* and *P. ficus* were obtained using the methods stated earlier. Therefore, there was no need to continue to design other methods for rearing. Although these methods produced sufficient numbers of insects for this study, further rearing methods would have to be considered for mass rearing of these insects. *Planococcus ficus* produced large amounts of honeydew, which resulted

in a significant presence of sooty mould on the butternuts. Starting new cultures by placing fresh butternuts on the lid of the cultures and allowing the first instar crawlers to move onto the butternuts appeared to reduce to spread of the sooty mould to the new cultures.

In order to rear insects sufficiently the temperature and humidity need to be controlled in the rearing environment. The first attempts to rear whitefly proved unsuccessful due to lack of environmental control. With the installation of the air-conditioning unit this problem was largely overcome and with increased ventilation in the rearing chambers clean cultures of whitefly were obtained. The rearing techniques for *S. oryzae* and *P. ficus* were not subjected to the conditions under wet wall cooling and therefore may not be adequate under those conditions.

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2.6   Appendix 2.1

(A)

(B)

Problems caused by conditions favouring pathogen development in the glass rearing tanks.

A, Diseased Eggplants and wilting from the high temperatures. B, Infected whitefly cadavers on a bean leaf.

CHAPTER 3

Susceptibility of four agricultural pests, *Sitophilus oryzae* (Lineaus) (Coleoptera: Curculioinidae), *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), *Planococcus ficus* (Signoid) (Hemiptera: Pseudococcidae) and *Eldana saccharina* Walker (Lepidoptera: Pyralidae) to various entomopathogenic fungi

Abstract

Sitophilus oryzae, Trialeurodes vaporariorum, Planococcus ficus and *Eldana saccharina* are four of the most agriculturally important pests occurring in Southern Africa. Several entomopathogenic fungi were initially tested against the four pests at 25°C (± 2°C) and 85% (± 2°C) relative humidity. Seven fungal isolates were tested against *S. oryzae*, four of which, B1, PPRI 6690, PPRI 6864 and PPRI 7062, caused >80% mortality over a 21d period and were selected for further testing. These four isolates were tested at varying conidial concentrations. No weevil mortality was recorded at the lower conidial concentrations of 1×10^3 and 1×10^4 conidia ml^{-1}. At higher doses (1×10^6 conidia/ml), mortality rates of up to 84% were recorded. LT_{50}'s for the four treatments ranged from 6-54d. *T. vaporariorum* was inoculated with 11 fungal isolates. Mean percentage mortality ranged from 27 - 75% over 14d. *Beauveria bassiana* Isolates B1 and PPRI 6690 caused >70% mortality and did not differ significantly from PPRI 6864 and PPRI 7062. Nine isolates were tested against *P. ficus*, however only two isolates, V1 and V2, resulted in low levels of control, with 23.4 and 6.7% mortality, respectively. Five isolates were tested against the second and third instar larvae of *E. saccharina*. Only Isolates B1, PPRI 6864 and PPRI 6690 resulted in mortality. Mean percentage mortality was low for all three isolates, ranging from 12 - 21% over 15d.

3.1 Introduction

Trialeurodes vaporariorum, Sitophilus oryzae, Planococcus ficus and *Eldana saccharina* are important pests of agricultural crops in South Africa. The rice weevil, *S. oryzae*, is one of the most serious storage pests of raw cereals throughout the world. The larval stages cause most of the damage, developing and feeding inside the seed. Synthetic chemical insecticides have been used to control these pests in a stored grain environment for the past 40 years. Because of growing public awareness to the adverse effects of these chemicals on both the consumer and the environment, many chemicals are no longer being used (Sheeba *et al.*, 2001). Various authors have demonstrated the use of entomopathogenic fungi to control a number of stored grain pests worldwide (Adane *et al.*, 1996; Moino *et al.*, 1998; Rice & Cogburn, 1999; Dal Bello *et al.*, 2001; Kassa *et al.*, 2002).

The greenhouse whitefly *T. vaporariorum* is found on many greenhouse vegetables and ornamentals (Byrne *et al.*, 1990). Damage to crops is mainly due to the production of honeydew produced by larval instars and feeding adults. This honeydew stimulates the growth of sooty mould both on the leaves and fruit, reducing both the photosynthetic capacity of the plant and market value of the crop. Whiteflies also serve as vectors for several economically important viral plant pathogens (Vidal *et al.*, 2003). Many chemicals have proven effective in the past, but since the 1970's growers have been confronted by many resistance problems to a number of insecticide classes (Gindin & Ben-Ze'ev, 1994). The use of fungi as control agents for whitefly in greenhouses has been addressed by various authors (Meade & Byrne, 1991; Chandler *et al.*, 1993; Vidal *et al.*, 2003; Feng *et al.*, 2004).

Planococcus ficus (grapevine mealybug) is a pest of South African vineyards and is dominant in the main vine growing areas of the Western Cape Province (Addison & Samways, 2000). The excretion of honeydew during feeding results in the growth of sooty mould reducing crop quality and yield (Walton *et al.*, 2004). The grapevine mealybug may also vector viral diseases (Engelbrecht & Kasdorf, 1990a; 1990b).

Control of this pest is achieved by proper sanitation practises and the use chemical applications.

Eldana saccharina is considered and important pest of sugarcane across southern Africa. The damage to the crop is as a result of the larvae boring into and feeding within the stems of the host plant (La Croix, 1992). Control of this pest is largely by good predictive modelling and the application of chemical insecticides. A literature search showed that the use of fungal pathogens to control both *E. saccharina* and *P. ficus* has not been addressed.

The purpose of this study was to test several entomopathogenic fungi for pathogenic activity towards *S. oryzae*, *T. vaporariorum*, *P. ficus* and *E. saccharina*.

3.2   Materials and methods

3.2.1   Insect cultures

Sitophilus oryzae, *T. vaporariorum* and *P. ficus* were reared as stated in Chapter 2. Second and third instar larvae of *E. saccharina* were obtained from Dr D. Conlong, South African Sugar Research Institute (SASRI), Durban.

3.2.2   Fungal isolates

Several entomopathogenic isolates were tested (Table 3.1). The cultures were maintained on PDA plates (potato dextrose agar) and incubated at 20°C (± 2°C). The conidia were obtained from 15d old cultures that were actively sporulating, by pipetting 10ml of a 0.05% Tween 80 solution onto the sporulating culture and agitating. The suspensions were vortexed for 1min to obtain an even spore distribution throughout the sample. The number of conidia was estimated using a haemocytometer and the conidial suspensions were adjusted to 1×10^3 and 2×10^6 spores per ml, respectively.

3.2.3 Sporulation and germination

The spores were harvested as stated above. Spore production was estimated using a haemocytometer. Three categories were established, ranging from no sporulation (-) to more than 1×10^6 (Table 3.1). Germination capacity of the spore suspensions was evaluated by spraying 1ml of spore suspension (standardized to 1×10^5 spores/ml) onto water agar plates using a hand held atomizer. The Petri dishes were incubated for 24hrs at 20°C. The percentage spore germination was evaluated by counting 100 spores per plate. For each isolate, three counts were performed using two plates on each occasion. The spore was rated as germinated when the germ tube was as long or longer than the width of the spore (Table 3.1).

Table 3.1 Complete list of fungal isolates tested against one or more of the following inset pests, *T. vaporariorum, S. oryzae, P. ficus* and *E. saccharina*.

Isolate		Fungal species	Host	Origin	Sporulation[1]	% Germination[2] ± SD
PPRI 6690	abcd	*Beauveria bassiana*	*Bombix mori* (Lepidoptera)	Bethlehem, Free State, South Africa	++	86.3 ± 2.6
PPRI 6688	ac	*B. bassiana*	***Diuraphis noxia*** (Homoptera)	Langgewens, Western Cape, South Africa	+	76.3 ± 5.0
PPRI 7061	a	*B. bassiana*	*D. noxia*	Bethlehem, Free State, South Africa	+	76.1 ± 3.4
PPRI 7062	abcd	*B. bassiana*	*D. noxia*	Bethlehem, Free State, South Africa	+	87.7 ± 0.8
PPRI 7064	d	*B. bassiana*	*D. noxia*	Bethlehem, Free State, South Africa	+	81.5 ± 10.2
B 1	abcd	*B. bassiana*	*T. vaporariorum* (Homoptera)	KwaZulu Natal, South Africa	++	91.1 ± 7.3
B 2	a	*B. bassiana*	Soil sample	KwaZulu Natal, South Africa	+	62.3 ± 1.0
V 7066	ac	*Lecanicillium lecanii*	Unknown	South Africa	+	63.8 ± 3.3
V 1	abc	*L. lecanii*	Unknown	South Africa	+	70.6 ± 1.1
V 2	ac	*L. lecanii*	*T. vaporariorum*	KwaZulu Natal, South Africa	++	62.4 ± 1.0
P1	ac	*Paecilomyces sp.*	Soil Sample	KwaZulu Natal, South Africa	++	87.3 ± 9.2
PPRI 6864	abcd	*P. farinosus*	*D. noxia*	Bethlehem, Free State, South Africa	++	92.0 ± 6.9
PPRI 7067	bc	*P. farinosus*	*D. noxia*	Bethlehem, Free State, South Africa	+	85.1 ± 2.8

[1] + = 1x105 – 1x10^6, ++ = > 1x10^6 conidia ml^{-1};

[2]Average percentage germination of conidia on water agar;

[a] Isolates tested against *T. vaporariorum*; [b] Isolates tested against *S. oryzae*; [c] Isolates tested against *P. ficus*; [d] Isolates tested against *E. saccharina*.

3.2.4 *Trialeurodes vaporariorum*

3.2.4.1 Bioassay procedure

To obtain second and third instar nymphs, 40 adult whiteflies were confined to 2cm clip cages (Website 1) on the abaxial surface of the bean plant leaves (1 clip-cage per leaf and two leaves per plant. The adult whiteflies were left for 36hrs to allow for sufficient oviposition. The adults and the cages were then removed from the plants. The development of the egg to second and third instar larvae took approximately 14 – 16d under the glasshouse conditions.

Three plants per treatment and two leaves per bean plant were used. The leaves containing the second and third instar larvae were sprayed with 2ml spore suspension containing 2×10^6 conidia/ml with a hand held atomizer. As a control a solution of 0.05% Tween 80 was used. The control treatment of 0.05% Tween 80 had no significant effect on whitefly in this trial. The leaves were strayed to a point of runoff. The plants were then moved into a dark dew chamber where the temperature was set to 25°C (±2°C) and the humidity to 85% (±2%). The plants were maintained under these conditions for 24hrs before being moved back into a glasshouse. The trial was maintained at 25°C (±2°C) and was subjected to environment humidity changes. The trial was run over a 14d period.

Assessment of the mortality took place 14d post inoculation. Nymph and adult whiteflies were considered infected if the fungus could be visibly seen on the nymph or adult, or if the insect was showing characteristic or behavioural changes such as visible colour changes. If there was any uncertainty about the cause of death a smear was made of the insect on a glass slide and observed under the microscope for hyphal growth. The fungi were reisolated from the infected insects to confirm the identification of the fungus and to maintain isolate virulence.

3.2.4.2 Statistical analysis

The trial design was a Completely Randomized Blocks design. A mortality count was used for the statistical analysis of the trial. Data collected from the experiment were subjected to one way Analysis of Variance and the individual means were compared using Fisher's Least Significant Difference test at the 5% level (GenStat: Version 7.1.0.198).

3.2.5 *Sitophilus oryzae*

The adult weevils (see Chapter 2) were collected from cultures by sieving the sorghum seed.

3.2.5.1 First-phase bioassay

Forty-five weevils were randomly selected from the sieve and placed into a Petri dish containing filter paper (9cm diameter). Seven treatments were applied with three replicates per treatment. The weevils were immobilized by placing the Petri dish into a 2l plastic container and subjecting them to CO_2 for 1min. The Petri dishes containing the anesthetized weevils were placed in a spray tower built by the Mechanical Instrument Workshop at the University of KwaZulu Natal, Pietermaritzburg, in accordance to the design published by Burgerjon (1956). Three ml of the 2×10^6 conidia/ml suspension was applied to the weevils with an airflow rate of 5l /min. As a control the weevils were sprayed with a 3ml solution of 0.05% Tween 80. To prevent cross contamination of the isolates the tower and nozzle was cleaned with 70% ethanol after each spore application. The weevils were kept without food for 36hrs at 25°C (± 2°C) and 80% (± 2%) relative humidity. They were then transferred to 250ml Erlenmeyer flasks filled with 50g of clean sorghum grain. Each flask was sealed with muslin cloth. The weevils were maintained in the dew chamber for the entirety of the experiment. Results were recorded 3d, 5d, 7d, 14d and 21d post inoculation by sieving the grain and collecting the dead

individuals. Dead individuals were surfaced sterilized by placing them in 70% ethanol for 1min and washing in sterile distilled water for 5min. The sterilized weevils were placed on moistened filter paper in sterile Petri dishes and maintained in an incubator at 20°C for 7d. Cadavers showing external hyphal growth resembling the characteristics of the fungal entomopathogen were recorded as infected and included in mortality analysis.

Data collected from the experiment were subjected to one way Analysis of Variance and the individual means were compared using Fisher's Least Significant Difference test at the 5% level. Lethal times required to kill 50% of the population (LT_{50}) was calculated using Probit analysis. A 95% confidence interval was set for the LT_{50}'s using Fieller's method (GenStat: Version 7.1.0.198).

3.2.5.2 Second-phase bioassay

Based on the results obtained from the initial screening process, four isolates resulting in the highest mortality, B1, PPRI 6690, PPRI 6864 and PPRI 7062, were selected. The treatments consisted of four concentrations and three replicates of each. The spore suspensions were adjusted to 1×10^3, 1×10^4, 1×10^5 and 1×10^6 conidia/ml respectively. The weevils were obtained from the stock culture and inoculated as stated above. Twenty weevils were used per rep per treatment. Dead insects were removed from the cultures on 3d, 5d, 7d, 14d and 21d. Dead individuals were treated exactly as in the first-phase bioassay.

Data collected from the experiment were subjected to two way Analysis of Variance and the individual means were compared using Fisher's Least Significant Difference test at the 5% level. LT_{50} data was calculated as above.

3.2.6 *Planococcus ficus*

Planococcus ficus was reared as stated in Chapter 2. Whole butternuts (*Cucurbita maxima*) containing the mealybugs were used. Thirty mealybugs (various life stages) were used per treatment with three reps for each. If the butternut contained more than thirty individuals the excess were removed before inoculation. Nine isolates were tested against *P. ficus* (Table 3.1). Spore preparation was prepared as above. The mealybugs were inoculated by placing the butternuts in a spray tower built by the mechanical instrument workshop at UKZN, Pietermaritzburg in accordance to the design published by Burgerjon, 1956 (Appendix 3.1). A 3ml spore suspension containing 1×10^6 conidia/ml was sprayed onto the mealybugs at a flow rate of 5l /min. The butternuts were then placed in a dew chamber and were maintained at 25°C (±2°C) with 85% (±2%) relative humidity for a period of 14d. As a control the mealybugs were sprayed with a 3ml solution of 0.05% Tween 80. The mealybugs were recorded as infected if the adults showed variation in colour and the fungal pathogen could be reisolated from cadavers. Overall mortality was used for the statistical analysis of the trial. A one way analysis of variance was used to determine significant differences between treatments (Fisher's Least Significant Different test at the 5% level).

3.2.7 *Eldana saccharina*

Second and third instar larvae were obtained form Dr. D. Conlong for use in the experiment. Five isolates were tested against *E. saccharina* (Table 3.1). Twenty larvae were used per treatment and there were three reps of each. Larvae were inoculated by placing Petri dishes containing twenty larvae into a Burgerjon spray tower and applying 3ml of 1×10^6 conidia/ml spore suspension at a flow rate of 5l /min. Spore preparation was prepared as stated earlier. As a control larvae were sprayed with a 3ml solution of 0.05% Tween 80. The growth media was then introduced into the Petri dishes. The inoculated larvae were then placed in a dew chamber and were maintained at 25°C (±2°C) with 85% (±2%). Results were recorded on 5d, 10d and 15d. The dead larvae were recorded as infected if the fungal pathogen could be reisolated from the cadavers. A mortality count was used for the statistical analysis of the trial. A one way analysis of

variance was used to determine significant differences between treatments (Fisher's Least Significant Different test at the 5% level).

3.3   Results

3.3.1   *Trialeurodes vaporariorum*

Nine isolates caused mortality in second and third instar larvae (Table 3.2). Two isolates, B2 and P1, did not infect the whitefly larvae or could not be reisolated from the diseased insects and therefore were excluded from the bioassay (Table 3.1). Virulence of the isolates differed significantly. Whitefly mortalities varied from 27 to 75%. Isolates B1 and PPRI 6690 resulted in >70% mortality over the 14d (Table 3.2). Isolates PPRI 7062 and PPRI 6864 caused mortalities of > 60% and did not differ significantly from B1 and PPRI 6690. The *Lecanicillium* isolates produced significantly lower levels of mortalities than *B. bassiana* and *P. farinosus*.

Table 3.2   Mean percentage mortality of *T. vaporariorum* after 14d. n = 100.

Treatment	Mean percentage mortality a		Standard error of the mean
B1	74.7	F	3.53
PPRI 6690	74.3	F	2.33
PPRI 7062	62.3	E	2.73
PPRI 6864	60.7	E	1.76
PPRI 7061	43.3	Bc	2.91
PPRI 6688	48.7	Cd	4.91
V 1	36.7	B	2.40
V 2	26.7	A	4.06
V 7066	47.7	Cd	1.86
Control	0		-
F test	28.2		
CV%	10.2		

[a] Values followed by the same letter do not differ significantly (Fisher's Least Significant Difference test, 5%).

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3.3.2   *Sitophilus oryzae*

3.3.2.1 First-phase bioassay
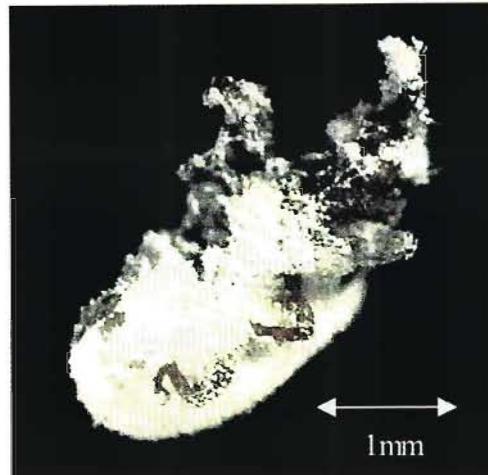
Figure 3.1   *Sitophilus oryzae* infected with *Beauveria bassiana* Isolate B1. Sporulation on the entomopathogen is evident (white mass) on the surface of the weevil.

Conidial growth on the cadavers indicated that all the isolates resulted in weevil mortality (Figure 3.1). The results showed a significant difference between the susceptibility of *S. oryzae* to the various fungal treatments (Table 3.3). Initial screening of *L. lecanii* and *Metarhizium* sp. resulted in no infection of the rice weevil and therefore were not considered for further testing in this bioassay. Mortality of *S. oryzae* ranged from 8% (PPRI 7067) to 81% (PPRI6690). Isolates B1, PPRI 6690, PPRI 7062 and PPRI 6864 resulted in the highest mortalities over the 21d period (Table 3.3). The LT_{50} values for the four treatments indicated rapid infection of *S. oryzae* ranging from 3.8 to 5.4d (Table 3.4).

Table 3.3   Mean percentage mortality in adult *Sitophilus oryzae* 21d post inoculation with a single rate of conidia of various entomopathogenic fungal strains.

Treatments	Exposure period in days[z]						Abbott's Corrected % Mortality
	3	5	7	14	21		
B1	28.1±6.5	64.5±8.2	80±4.4	80.8±5.6	81.5±4.6	cd	77.48
PPRI 7062	28.2±9.2	48.1±8.4	73.3±8.0	78.5±8.9	80.0±8.0	cd	75.68
PPRI 7064	11.9±5.6	42.9±10.0	63.0±12.8	64.5±13.5	65.9±7.1	b	58.56
PPRI 6864	21.5±13.4	46.7±17.8	76.3±6.4	77.7±5.8	80.7±2.5	cd	76.58
PPRI 7067	2.2±2.2	7.4±9.0	13.3±5.9	17.0±8.4	24.5±10.2	a	8.11
PPRI 6684	17.0±5.6	42.9±5.6	61.5±5.6	67.4±5.6	62.9±6.8	bc	63.96
PPRI 6690	28.9±8.0	66.7±2.3	74.8±11.4	80.0±2.2	83.7±3.4	d	80.80
Control	0.7±1.3	2.2±2.2	8.2±2.5	11.1±2.2	17.8±2.2		
F test					22.2		
CV%					11.0		

[z] Values are mean % mortality ± standard deviation

Means followed by the same letter do not differ significantly (LSD) at 5% level.

Table 3.4 LT_{50}'s for the screening of seven entomopathogenic fungi.[a]

Treatment	LT_{50}	95% Confidence interval		χ^2
		Lower	Upper	
B1	3.972	3.175	4.7	9.818
PPRI 7062	5.052	4.249	5.825	50.97
PPRI 7064	8.089	6.987	9.389	34.53
PPRI 6864	5.453	4.716	6.18	63.42
PPRI 7067	69.663	41.305	192.75	55.21
PPRI 6684	6.96	5.984	8.019	27.81
PPRI 6690	3.848	3.093	4.536	38.84

3.3.2.2 Second-phase bioassay

Based on the results from the first-phase bioassay, Isolates B1, PPRI 6690, PPRI 6864 and PPRI 7062 were selected for further testing. Results showed little or no infection at the lower conidial concentrations of 1×10^3 and 1×10^4 conidia ml^{-1}. As above, the mortality of *S. oryzae* was incremental. Treatments with B1 and PPRI 6690 produced the highest mortality at 1×10^6 conidia ml^{-1}, both resulting in 68% mortality of the population after 21d . LT$_{50}$'s were calculated at 6.8 and 5.9d, respectively (Table 3.5).

Table 3.5　Concentration data showing LT_{50}'s (mean and 95% confidence intervals) for four isolates tested against *S. oryzae*.

Treatment	Concentration (conidia ml^{-1})	% Mortality	95% Confidence interval	LT_{50}[a]		95% Confidence interval	χ^2	P Value
Control		8.33	(0-20.4)	180.3	abc	*		
B1	1x10^3	3.3	(0-11.2)	-		*	16.08	0.0973
	1x10^4	28.3	(8.6-48.1)	33.6	ef	(17.9-321.4)	10.92	0.3635
	1x10^5	40	(18.5-61.5)	18.9	g	(12.6-54.9)	6.595	0.7630
	1x10^6	68.3	(47.9-88.7)	6.8	h	(5.4-8.8)	4.736	0.9081
PPRI 6690	1x10^3	-	-	-		*	11.31	0.3339
	1x10^4	18.3	(1.37-35.3)	51.5	cde	(23.3-1538)	7.037	0.7220
	1x10^5	40	(18.5-61.5)	20.7	g	(12.6-116.7)	13.31	0.2068
	1x10^6	68.3	(47.9-88.7)	5.9	h	(4.4-7.7)	10.85	0.3695
PPRI 6864	1x10^3	5	(0-15.5)	-		*	9.946	0.4453
	1x10^4	15	(0-30.6)	52.7	bcd	(24.2-1438)	5.331	0.8680
	1x10^5	28.3	(8.6-48.1)	36.8	ef	(17.9-948.4)	6.002	0.8151
	1x10^6	63.3	(42.2-84.5)	7.8	h	(6.1-11.2)	8.049	0.6241
PPRI 7062	1x10^3	1.67	(0-7.3)	-		*	2.231	0.9942
	1x10^4	21.67	(3.6-39.7)	67.9	de	(23.9-1620)	10.08	0.4331
	1x10^5	25	(6.0-43.9)	42.6	de	(20.8-679.2)	3.897	0.9519
	1x10^6	36.7	(15.6-57.8)	25.3	fg	(13.8-338)	2.321	0.9932
F test (treatment)		4.71						
F test (concentration)		90.35						
CV%		34.5						

* Confidence intervals not calculated because the variable time was not significant at the 5% level of significance.

[a] Values followed by the same letter in the same column do not differ significantly (LSD, at the 5%level).

3.3.3 *Planococcus ficus*

Nine isolates were tested, two of which caused mortality in *P. ficus* (Figure 3.2). The mealybugs were considered infected if adults showed variation in colour and the pathogen could be reisolated from the cadaver. Significant differences between treatments were observed. Isolate V2 caused 23% mortality and V1 resulted in 7% (Figure 3.2).
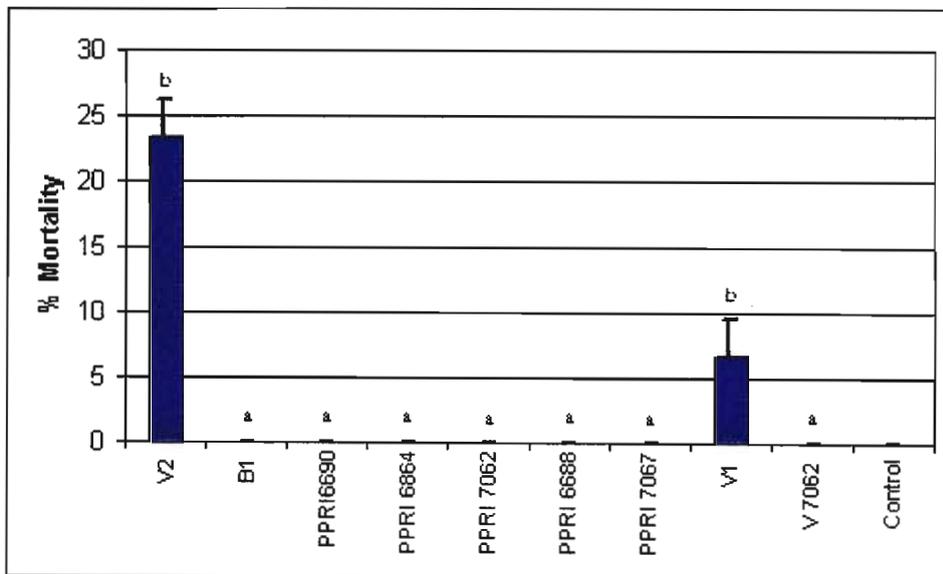
Figure 3.2   Mean percentage mortality of *Planococcus ficus*. Error bars represent standard deviation. F test = 99.98, CV% = 43.0%. Bars not followed by the same letter are significantly different (LSD, at 5% level).

3.3.4 *Eldana saccharina*

In the study, five fungal isolates were tested against second and third instar larvae, three of which resulted in mortality. Isolates B1, PPRI 6690 and PPRI 6864 caused mortalities ranging from 11.6% for Isolate B1 to 21.4% for Isolate PPRI 6864 (Figure 3.3).
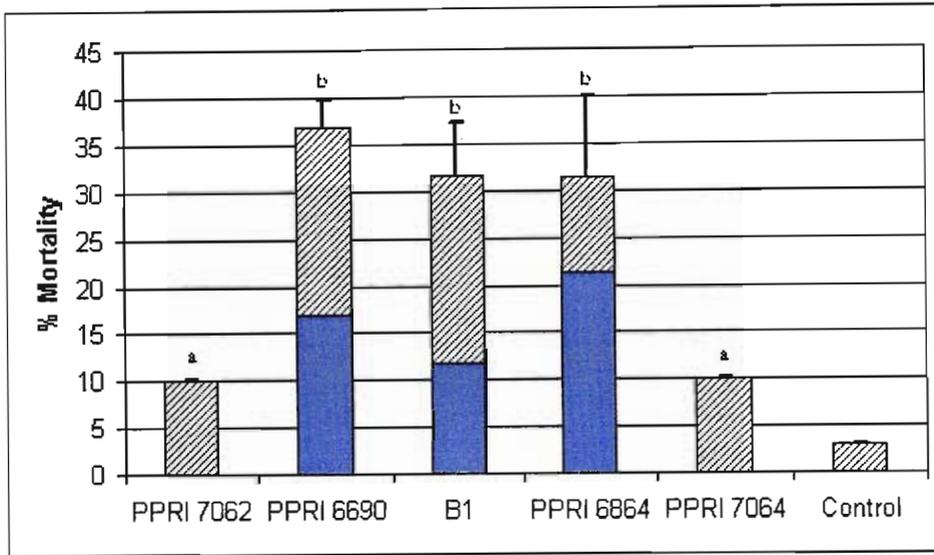
Figure 3.3   Mean percentage mortality of *Eldana saccharina* (blue bars) and other causes (shaded bars). Other causes represent dead insects not infected by the entomopathogen. Error bars represent standard deviation. F test = 16.95, CV% = 44.0%. Bars not followed by the same letter are significantly different (LSD, at 5% level).

3.4   Discussion

3.4.1   *Trialeurodes vaporariorum*

Whiteflies are important pests of greenhouse crops and are known to vector economically important viral diseases. Due to resistance problems alternative means of control are being investigated. From this study it was found that nine isolates caused mortality in second and third instar larvae (Table 3.2). The whitefly nymphs were considered infected if the fungus was actively sporulating on the surface of the nymph or the insect was showing characteristic colour changes (Appendix 3.2). Three groups of isolates could be established: those that did not infect the whitefly, an intermediate group (<50% mortality) and a group that performed well (>50% mortality). Four of the nine isolates tested produced mortality of above 60%. It can not be disputed that fungal biological control agents are not as affective as chemical control based solely on the short-term crop-

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production economics (Faria & Wraight, 2001). Chemical control is easy to apply, fast acting and widely available. Control of above 60% as in this study would be considered by most to represent a significant level of whitefly suppression. However it is simply not competitive with an insecticidal application which is capable of providing 100% control of the pest population at a lower cost (Faria & Wraight, 2001). However, it is believed that fungal biological control agents do have their place and are relevant to whitefly control especially considering organic farming. The use of this method of control in an IPM program could reduce the likelihood of pests developing resistance to chemical applications and therefore has the potential to extend the market life of chemical insecticides. Further studies into the performance of Isolates B1, PPRI 6690, PPRI 7062 and PPRI 6864 are needed in order to get a better insight into their speed of kill (LT_{50}) and the mortality effects of spore concentration, temperature and humidity levels. These are critical characteristics when one is selecting for biological control agents.

3.4.2 *Sitophilus oryzae*

3.4.2.1 First-phase bioassay

The results obtained in this study are in accordance to reports by Moino *et al.* (1998) and Dal Bello *et al.* (2001), which indicated greater mortalities of stored grain pests inoculated with *B. bassiana* than with *Metarhizium anisopliae*. Mortality of *S. oryzae* ranged from 8% - 81% and the results were in accordance to those obtained by Moino *et al.*, (1998). They tested various *B. bassiana* treatments against three storage grain pests and obtained mortality of *S. oryzae* ranging from 50 - 90%. Treatments B1, PPRI 6690, PPRI 7062 and PPRI 6864 produced the highest mortalities and the LT_{50} values indicated rapid infection of *S. oryzae* which is an important characteristic when selecting fungal isolates as potential biological control agents (Tanada & Kaya, 1993). Therefore, this was used as a major characteristic when selecting isolates for further testing in the second-phase bioassay. These four treatments not only produced significant infection of *S. oryzae* but also grew rapidly

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in culture and produced high levels of sporulation and germination. Subsequently they were selected for further testing.

3.4.2.2 Second-phase bioassay

Early reports indicated that there is an increase in rice weevil mortality with increase conidial concentration (Adane *et al.*, 1996; Rice & Cogburn, 1999; Sheeba *et al.*, 2001). The present study agrees with the reports of higher conidial concentration causing higher mortality rates in *S. oryzae* populations. Treatment B1 and PPRI 6690 produced the highest mortality at 1×10^6 conidia ml^{-1}. Conidial concentration of less than 1×10^5 conidia ml^{-1} produced low levels of mortality for all the isolates tested. Based on the results of the second-phase bioassay treatments B1 and PPRI 6690 may be considered as potential biological control agents of *S. oryzae* and warrant further study. Research into mass-production, yields and specificity need to be considered before recommending any of the isolates as possible biological control agents. Application of fungal isolates to large quantities of stored grain possesses many problems. The stored grain environment is not conducive to fungal infection of insect pests. Grain is generally stored with a low water content producing an environment of low humidity. Generally fungi need high levels of humidity to infect target organisms. Further studies into microclimates within stored grain would give some insight as to whether this method of control works in practise.

An integrated approach to controlling pest in stored grain may prove to be the most effective approach in the future. Dal Bello *et al.* (2001) suggests that biological control should be compatible with other control practices such as the use of insecticides. Their results indicated an increase in *S. oryzae* mortality when *B. bassiana* was applied with a chemical control measure. Progress must still be made to improve reliability and the effectiveness of biological control agents before entomopathogenic fungi are used as a control method for storage grain pests.

3.4.3 *Planococcus ficus*

Nine isolates were tested, only two of which caused mortality in *P. ficus* (Figure 3.3). The mealybugs were considered infected if adults showed variation in colour and the pathogen could be reisolated from the cadaver. Significant differences between treatments were observed. Isolate V2 caused 23% mortality and V1 resulted in 7% (Figure 3.3). *Planococcus ficus* is a cryptic pest and spends part of its life cycle under the bark of the grapevines during the winter months. Therefore, this produces an ideal micro-climate favouring the infection process of entomopathogenic fungi. With an effective application method, a virulent pathogen strain and the use of an adjuvant such as 'Breakthru', the use of biological control may be a viable control option. Although the mortality rates were low in this study, only a small number of *Lecanicillium* isolates were tested against *P. ficus*. With further testing of various fungal strains and application methods the use entomopathogenic fungi as a biological control agent of *P. ficus* may become a reality, limiting the need for insecticidal applications.

3.4.4 *Eldana saccharina*

Eldana saccharina causes substantial losses in sugarcane. The control of this pest has, in the past, been achieved with the use of chemical applications. However, new methods for control are being investigated. Conlong (1994) discussed the interactions between *Eldana* and various parasitoids and their potential as biological control agents. The literature indicates that fungal entomopathogens have been found to infect the larval stages of *E. saccharina*; however, no further research has been conducted into the potential of these fungi. The results from this study indicated that there was minimal control of *E. saccharina*. Mortalities of less than 20% were recorded. A number of cadavers had the characteristics of infection. However, the fungus could not be reisolated and therefore could not be recorded as infected. Although the mean percentage mortality was low for the respective treatments, there is a potential for further research and screening of different entomopathogenic fungi against *E. saccharina*. Due to the cryptic nature of this pest, targeting the various life stages for control is difficult. With fungal biological control one could only target the fist to third instar larvae and the adults, as these are the

only life stages outside of the stem. Therefore it would be critical to have a good monitoring system so as to be able to apply the conidial application at the optimum time. Further investigation into the affect of various entomopathogenic fungi on the 1st instar larvae should be considered as well as the use of walk through traps to target the adult moths suppressing the potential of future populations.

3.5   Conclusion

Entomopathogenic fungi and insect natural enemies have the potential to complement or interfere with one another, depending on the environmental conditions (Lacey *et al.*, 1977). The use of an integrated control program for the control insect pests may limit the use of chemical applications. A good integrated control program incorporates a good monitoring program and the timeous inoculation of fungal entomopathogens and release of parasitoids. However, further research is required before the full potential of an integrated control program may be realized.

3.6 References

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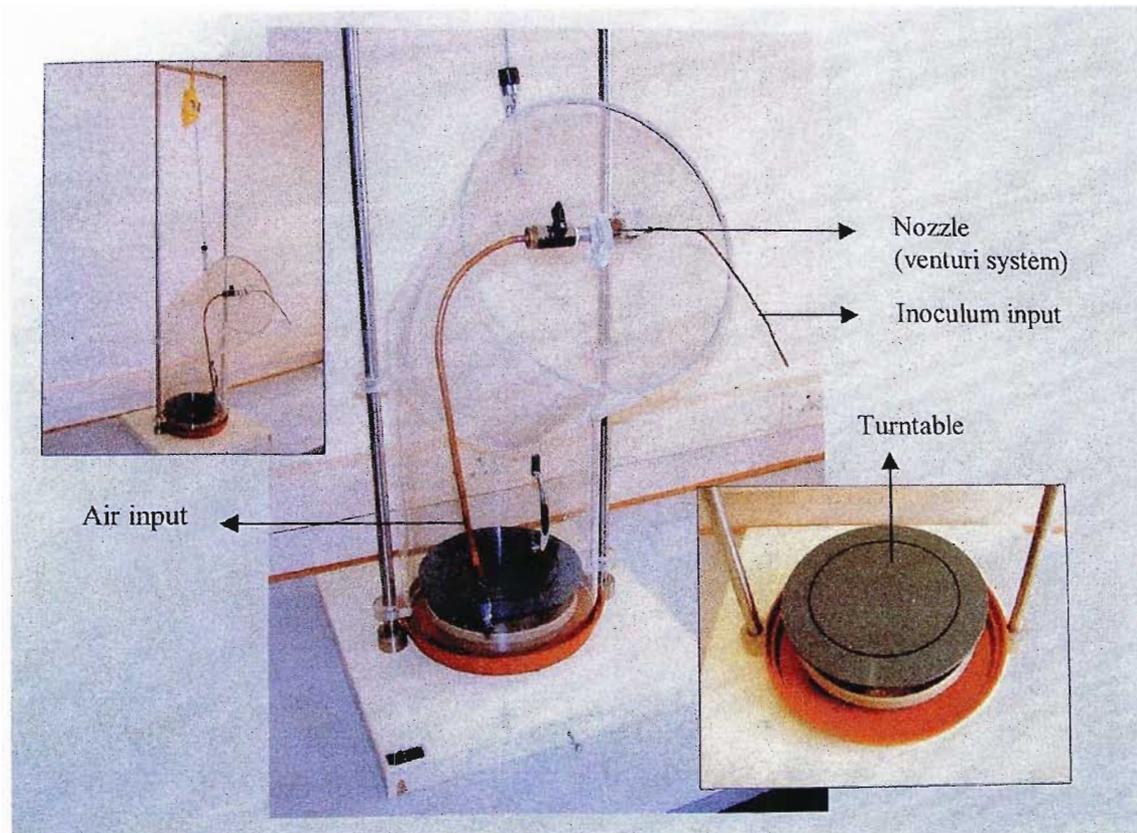
3.6 Appendix 3.1

Burgerjon Spray Tower (Hatting, 2002)

Appendix 3.2

(i)

(ii)

(i) *Trialeurodes vaporariorum* infected with *B. bassiana* (PPRI 6690) showing characteristic colour changes, (ii) second and third instar nymphs infected with *B. bassiana* (PPRI 6864). Healthy nymphs appear clear to transparent.

Chapter 4

Effect of temperature and humidity on the susceptibility of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) to four entomopathogenic fungi

Abstract

The effect of temperature and relative humidity on the pathogenicity of four entomopathogenic isolates to the rice weevil *Sitophilus oryzae* was examined. The highest mortality rates were obtained at 25°C where mortality ranged from 46 - 65% after 14d. The relative humidity was maintained at 85% for the duration of the temperature trials. Mortality rates decreased with decreasing temperature, however no mortality was recorded for all four isolates at 30°C. Temperature altered the LT_{50} significantly, with Isolate PPRI 6690 producing the lowest LT_{50} of 11.8d at 25°C. The optimum temperature for all four isolates was 25°C. Constant relative humidities were maintained using saturated salt solutions at 25°C. Decreasing the humidity resulted in an increased time taken to kill 50% of the population (LT_{50}) and a reduction in the overall mortality rates. The mortality of *S. oryzae* ranged according to the relative humidity and isolate. Isolates B1 and PPRI 6690 resulted in mortalities of 80 and 83.3% at 92.5% RH, with LT_{50}'s of 6.3 and 6.4d, respectively. Both temperature and relative humidity were found to significantly affect the infection potential of the isolates. Increased humidity and temperature generally resulted in an increase in mortality. Taking into account both the temperature and humidity results, Isolates B1 and PPRI 6690 showed the most potential for further development as biological control agents of *S. oryzae*.

4.1 Introduction

The rice weevil, *Sitophilus oryzae* (L.), is a cosmopolitan species found worldwide. It is one of the main storage grain pests in South Africa and attacks a variety of other grains as well as processed products such as macaroni. Most damage to storage grain occurs when conditions are favourable to their development (25-35°C and a low relative humidity). The susceptibility of stored grain pests to fungal biological control agents has been demonstrated by various authors (Rice & Cogburn, 1999; Sheeba *et al.*, 2001, Kassa *et al.*, 2002, Batta, 2003). Results have demonstrated mortalities of >80% by entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin, *Lecanicillium lecanii* (Zimm.) Zares & Gams and *Metarhizium* sp. Development of successful mycosis in an insect depends on the adhesion of the fungal spore to the insect's integument, the germination of the spore and the penetration into the haemocoel. Spore germination largely depends on the environmental temperature and humidity, and to some extent, varying light conditions. It has long been considered that fungal epidemics depend largely on specific weather conditions; usually they are associated with high humidities and average temperatures (Ferron *et al.*, 1991, Tanada & Kaya, 1993). The conditions under which the fungi are capable of optimum infection must be similar to those found in the environment of the pest. If this were not the case, then minimal infection of the pest would occur, and there would be no economic justification for the application of the biological control agent.

The aim of this study was to assess the influence of both temperature and humidity on the infection potential of four *B. bassiana* strains to control the rice weevil, *S. oryzae*.

4.2 Materials and methods

4.2.1 Source and rearing of *Sitophilus oryzae*

S. oryzae was reared and maintained as stated in Chapter 2.

4.2.2 Fungal isolates

Four isolates B1, PPRI 6690, PPRI 6864 and PPRI 7062 were selected from the results obtained in Chapter 3. Spore preparations were conducted as stated in Chapter 3. The number of conidia was estimated using a haemocytometer and the conidial suspensions were adjusted to 1×10^6 conidia/ml.

4.2.3 Humidity bioassay

Weevils were collected from the cultures by sieving sorghum grain. Twenty adult weevils were randomly selected from the sieve and placed into Petri dishes containing filter paper (9cm diameter). Four treatments were applied, three replicates per treatment. The weevils were gassed with CO_2 until all the individuals appeared immobile. This was achieved by placing the Petri dishes, containing the weevils, into a 2l plastic container then filling it with CO_2 and replacing the lid. The weevils were then monitored until all the individuals appeared immobile. The Petri dishes containing the anesthetized weevils were placed in a Burgerjon spray tower built by the UKZN, Pietermaritzburg, in accordance, with the plans of Burgerjon (1956). Three ml of the conidial suspension was applied to the weevils. For the control treatments, the weevils were sprayed with a 3ml solution of 0.05% Tween 80. Twenty grams of clean sorghum seed was placed into a clean Petri dish and the inoculated weevils added. The inoculated Petri dishes were placed into 2l plastic containers, which were incubated at 25°C in a standard laboratory incubator. The humidity was adjusted in the plastic containers by adding saturated salt solutions. The solutions were made up according to Wilson & Bates (1960). The humidities

were adjusted to 92.5, 85, 76, 62 and 55%, respectively. Humidity levels were verified by placing a Hobo into 2l containers containing the salt solutions.

Results were recorded 3d, 5d, 7d, 14d and 21d post inoculation, by sieving the grain and identifying the dead individuals. The dead individuals were surface sterilized by placing them in 70% ethanol for 1min and washing in sterile distilled water for 5min. The sterilized weevils were placed on moistened filter paper in sterile Petri dishes and maintained in an incubator at 25°C for 7d. Cadavers showing external hyphal growth resembling the characteristics of the fungal entomopathogen were recorded as infected.

4.2.4 Temperature bioassay

The weevils were selected and inoculated as above. The humidity was adjusted to 85% with the use of saturated salt solutions (Wilson & Bates, 1960). The plastic containers were incubated at 15, 20, 25 and 30°C. Dead insects were removed from the Petri dishes 3d, 5d, 7d, 14d and 21d post inoculation, by sieving the grain and identifying the dead individuals. Mortality was assessed as stated above.

4.2.5 Statistics

The trial design was a Completely Randomized Blocks design. The data was analyzed using Probit analysis and for the different treatments the lethal times for 50% (LT_{50}) of the weevils to be killed was obtained. 95% confidence intervals for the LT_{50}'s were calculated using Fieller's method. An analysis of variance (ANOVA) was used to determine any significant differences between treatments, using a 5% level of significance.

4.3 Results

4.3.1 Humidity bioassay

Five relative humidities (rH) were tested for their effect on the infection potential against *S. oryzae* at 25°C. Infection was recorded at all five rH tested, with a significant difference between the treatments. Total mortality declined with reducing rH in all five treatments. Treatments B1 and PPRI 6690 resulted in a mean percentage mortality of >80% at 92.5% rH and LT_{50}'s were 6.4 and 6.3d, respectively (Table 4.1). The speed of kill reduced significantly with a reduction in rH.

Table 4.1 Percentage mortality (mean ± SD), and LT_{50}'s (95% confidence) for four *Beauveria bassiana* strains tested against *Sitophilus oryzae* at varying relative humidities.

Treatment	Relative Humidity (%)	% Mortality ± SD [a,b]		LT_{50} (days)
B1	55.0	21.6 ± 9.7	b	70.95
	62.0	28.33 ± 11.1	bcd	52.84
	76.0	35.0 ± 11.8	cde	48.41
	85.0	45.0 ± 13.2	fg	26.4
	92.5	80.0 ± 21.1	i	6.40
PPRI 6690	55.0	21.3 ± 7.9	b	99.08
	62.0	40.0 ± 11.8	efg	39.26
	76.0	65.0 ± 18.1	h	15.45
	85.0	42.0 ± 21.8	h	11.30
	92.5	83.3 ± 4.3	i	6.29
PPRI 6864	55.0	28.3 ± 10.7	bcd	70.15
	62.0	25.0 ± 9.6	b	108.89
	76.0	36.7 ± 10.2	def	47.75
	85.0	46.7 ± 16.2	g	21.42
	92.5	63.3 ± 8.3	h	11.56
PPRI 7062	55.0	21.7 ± 8.8	b	114.23
	62.0	26.7 ± 10.7	bc	67.30
	76.0	35.0 ± 9.1	cde	47.64
	85.0	53.2 ± 13.1	efg	36.06
	92.5	61.7 ± 8.8	h	14.59
Control	55.0	11.6 ± 7.5	a	-
	62.0	5.6 ± 9.8	a	-
	76.0	7.8 ± 8.3	a	-
	85.0	5.6 ± 6.3	a	-
	92.5	6.6 ± 7.5	a	-
F test (treatment)		35.93		
F test (humidity)		39.07		
CV%		27.0		

[a] Values are mean % mortality ±standard deviation of three replicates.

[b] Values followed by the same letter in the same column do not differ significantly (LSD, at the 5% level).

4.3.2 Temperature bioassay

The four *B. bassiana* treatments resulted in mortality of *S. oryzae* at all but the highest temperature of 30°C (Table 4.2). The rH was maintained at 85% (± 2%) for the entirety of the experiment so as to optimize infection conditions with the use of different salt solutions (Wilson & Bates, 1960). Mortality of *S. oryzae* ranged from 15 - 41% over the 14d period. The highest mortalities were obtained at 25°C, and ranged from 47 - 65%. The LT_{50}'s were calculated using Probit analysis and results varied from 11.8 - 33d at 25°C. PPRI 6690 produced the highest mean percentage mortality (65%) and the lowest LT_{50} (11.8d) (Table 4.2).

Table 4.2 Percentage mortality (mean ± SD), and LT_{50}'s (95% confidence) for four *Beauveria bassiana* strains tested against *Sitophilus oryzae* at varying temperatures.

Treatment	Temperature (°C)	% Mortality ± SD [a][b]		LT_{50} (days)
B1	15	23.3 ± 11.3	b	126.2
	20	29.7 ± 13.7	cd	26.6
	25	46.6 ± 14.1	cd	24.4
	30	-		-
PPRI 6690	15	25.6 ± 9.2	b	93.8
	20	55.0 ± 7.1	de	15.4
	25	65.0 ± 9.1	e	11.8
	30	-		-
PPRI 6864	15	21.7 ± 9.1	b	157.8
	20	51.7 ± 16.2	cd	20.1
	25	60.0 ± 15.9	cd	20.4
	30	-		-
PPRI 7062	15	23.3 ± 7.0	b	205.6
	20	53.3 ± 17.6	cd	19.5
	25	53.3 ± 10.6	c	33.0
	30	-		-
Control	15	3.3 ± 3.5	a	-
	20	4.6 ± 5.2	a	-
	25	2.8 ± 3.6	a	-
	30	5.5 ± 5.8	a	-
F test (treatment)		48.54		
F test (temperature)		10.86		
CV%		41.2		

- No data was obtained at 30°C due to lack of fungal infection at this temperature.

[a] Values are mean % mortality ±standard deviation of three replicates.

[b] Values followed by the same letter in the same column do not differ significantly (LSD, at the 5% level).

4.4   Discussion

Temperature and humidity affect the infection capabilities of a fungus. Each entomopathogenic fungus has its own optimum conditions under which infection is maximized. Generally the optimum temperature for growth of a fungus coincides with the optimum condition for infection (Meekes, 2001). The weevils were recorded as infected if the fungus was actively sporulating on the surface of the insect after surface sterilization and incubation of the cadavers at 25°C (Figure 4.1).

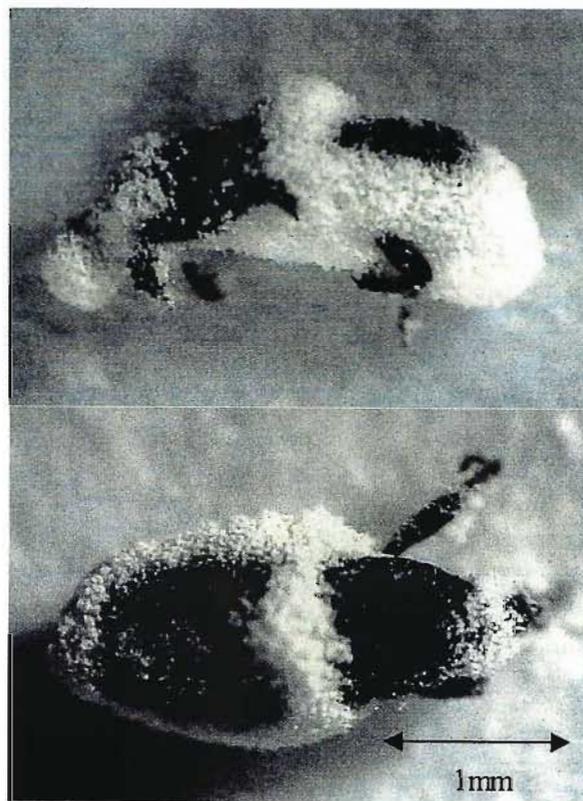Figure 4.1   Isolate PPRI 6690 actively sporulating (white mass) on the surface of *S. oryzae.*

Spore germination depends mainly on the presence of favourable environmental conditions. These conditions include high rH, usually greater than 90%, optimum temperatures and to a lesser extent light conditions (Tanada & Kaya, 1993). The humidity of the microenvironment surrounding the spore is, however, more influential than the

ambient rH. Therefore it is possible for *B. bassiana* to germinate on the surface of the cuticle even though the ambient rH is below 90% (Website 1). Hluchy & Samśiňáková (1989) reported that when the relative humidity dropped below 90%, the impact of spore concentration was reduced to a minimum. Therefore, having the correct environmental conditions while applying an entomopathogen may allow one to apply a lower conidial concentration to a pest population but still maintain a high mortality rate. Both temperature and rH humidity were found to significantly affect the infection potential of *B. bassiana* against *S. oryzae* during this study. Increased rH and temperature generally resulted in an increase in mortality.

The mortality rates for the trial were low in comparison with those reported in Chapter 3 and can be accounted for by considering the overall germination rate. The germination rate of the fungal spores was tested for each treatment at 20°C. The results showed a reduction in the germination percentage of the four treatments being tested. This could not be explained as the same experimental procedures were followed as in Chapter 3. The germination percentage was, however, similar for all the treatments and ranged from 65 - 70%. Germination at 30°C was <15% for all four treatments. The lack of growth of the *B. bassiana* cultures at 30°C was unexpected. Roberts & Yendol (1981) indicated that *B. bassiana* has a wide thermal tolerance and that growth of the fungus may occur from 5 - 35°C, with the optimum temperature being between 20 and 30°C. Because the experiment was conducted over a 14d period, mortality may have been underestimated. By extending the trial to 21d less extrapolation of the data sets would have occurred, resulting in more accurate results.

The mortality results obtained in this study at 25°C are similar to those published by Dal Bello *et al.* (2001) and Sheeba *et al.* (2001) who recorded mortalities ranging from 24 - 50% over a 14d period. From the results it is evident that temperature affects the infection potential of *B. bassiana* on *S. oryzae*. There was an increase in mortality with an increase in temperature. However, at 30°C infection potential of the fungus appeared to be severely retarded. This indicates that the optimum temperature of infection of *S. oryzae* by *B. bassiana* is near 25°C. Ferron (1978)

indicated that the optimum temperature of *B. bassiana* was 25°C, which is in accordance with the result of this study.

Taking into account both the temperature and humidity, Isolates B1 and PPRI 6690 showed the most potential for further development as biological control agents of *S. oryzae*. Integrated management of insect pests is considered the most effective approach for control. Further research is needed in order to assess the effectiveness of these two isolates in a possible integrated control program.

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CHAPTER 5

Thesis overview

5.1   Introduction

In recent years the value of biological control has been recognised as a viable practice of pest control. People are becoming aware of the potential ecological damage caused by chemical applications and are therefore putting pressure on growers to produce crops in an environmentally friendly way. In an undisturbed system most pests have natural enemies, such as predators, parasitoids and pathogens. Successful biological control depends on the ability to manipulate the natural enemies of the pest by various means, to maintain the pest population at levels below the economic threshold of the crop. With biological control, total eradication of the pest population is seldom possible (Burge, 1988).

Fungal biological control agents are one of the most interesting and exciting means of insect control. It has been known for a number of years that insects may be infected and killed by entomopathogenic fungi, either individually or through epizootics. Many chemical control measures have proven effective in the past but since the 1970's growers have been confronted by many resistance problems to a number of insecticide classes. This has resulted in a search for alternative measures to control insect pests. Research has shown that a number of fungal entomopathogens infect and control pest insect populations (Adane *et al.*, 1996, Moino *et al.*, 1998, Rice & Cogburn, 1999, Dal Bello *et al.*, 2001, Sheeba *et al.*, 2001, Kassa *et al.*, 2002, Batta, 2003). Some of the more common fungi known to infect insect populations include *Beauveria bassiana*, *Lecanicillium lecanii*, *Metarhizium anisopliae* and *Paecilomyces fumosoroseus*.

This study looked at the potential for entomopathogenic fungi to infect and control four important pests of South African agriculture. The four pests included the

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greenhouse whitefly, *Trialeurodes vaporariorum*, the rice weevil, *Sitophilus oryzae*, the grapevine mealybug, *Planococcus ficus* and a sugarcane borer, *Eldana saccharina*.

5.2   Problems associated with fungal biological control

Fungal biological control is affected by prevailing environmental conditions after spore application. Fungi require high levels of humidity before spore germination can take place, therefore field conditions need to meet the fungal requirements before infection of the pest population can take place. Large scale application of fungal biocontrol agents requires artificial production of the active agent. Therefore, the production of inoculum should be relatively easy and the product storage qualities should be good. However, research has shown that problems with formulation and the maintenance of fungal strains exists, such as gaining consistent control, slow killing action and limited shelf life.

Further research is needed into formulations that reduce the fungal pathogens reliance on high levels of humidity. If this is not obtained, the potential for the use of fungi as biocontrol agents will never be realised. Agriculture would benefit from the advantages of prolonged pest control, reduced risk of resistance and a greater degree of consumer safety (Burge, 1988). However, the commercialisation of biological control products depends largely on economics (Roberts & Yendol, 1981).

5.3   Fungal biological control of agricultural pests

5.3.1   *Trialeurodes vaporariorum*

The greenhouse whitefly, *T. vaporariorum*, is found on many greenhouse vegetables and ornamentals (Byrne *et al.*, 1990). Damage to crops is mainly caused by the production of honeydew produced by larval instars and feeding adults. Whiteflies also serve as vectors for several economically important viral plant pathogens (Vidal *et al.*, 2003). Chemical control has been used in the past to effectively control this

pest, however, due to the development of resistance in whitefly, other forms of control have recently been addressed. Several entomopathogenic fungi were screened against *T. vaporariorum*. Mean percentage mortality was found to range from 26.7-74.7% over 14d. Isolates B1 and PPRI 6690 caused >70% mortality. Due to the condition usually found in a glasshouse the use of fungal biological control is a viable proposition. Quinlan (1986) reported that temperatures are generally moderate and the humidity levels are high in glasshouses, and with slight adjustments the conditions could be altered to favour entomopathogenic fungi.

5.3.2 *Sitophilus oryzae*

The rice weevil, *Sitophilus oryzae* is considered one of the most serious pests of raw cereals throughout the world. Storage grain pests result in 10% loss of grains worldwide (Moino *et al.*, 1998). The larval stages of the rice weevil cause the majority of the damage. Damage to the grain may be both qualitative and quantitative (Moino *et al.*, 1998). Quantitative damage is due to grain weight loss as the larvae consume approximately 30% (by weight) of the kernel, feeding mainly on endosperm (Smit, 1964, Wilber & Mills, 1985). Qualitative damage includes the loss of nutrition and aesthetic value of the stored seed (Moino *et al.*, 1998).

Various authors have reported mortalities in *S. oryzae* as a result of inoculation with entomopathogenic fungi (Adane *et al.*, 1996; Moino *et al.*, 1998; Rice & Cogburn, 1999; Dal Bello *et al.*, 2001; Kassa *et al.*, 2002). This thesis looked at screening and selecting entomopathogenic fungi for control of *S. oryzae*. Four isolates B1, PPRI 6690, PPRI 6864 and PPRI 7067 were selected for further testing as each isolate caused >75% mortality of *S. oryzae* adults over a 21d period. These isolates were subjected to concentration, temperature and humidity bioassays. Higher conidial concentrations resulted in an increase in mortality. Mortality rates decreased with decreasing temperature, however no mortality was recorded for all four isolates above 30°C. Temperature was also found to alter the LT_{50}'s significantly. The

optimum temperature for all four isolates was 25°C. Relative humidity significantly affected the mortality of *S. oryzae*, decreasing with a reduction of humidity.

Increased humidity and temperature generally resulted in an increase in mortality. Isolates B1 and PPRI 6690 showed the most potential for further development as biological control agents of *S. oryzae*. Implications of this study for the farmer are that conditions of average temperature and high relative humidity are essential to produce satisfactory levels of control. However, grain is usually stored with a low moisture content therefore resulting in low levels of humidity in the storage areas. Therefore without a formulation that enables the entomopathogen to infect at lower humidity levels, this form of control in stored grain will not be a viable option.

5.3.3 *Planococcus ficus* and *Eldana saccharina*

The grapevine mealybug, *P. ficus*, is a key pest of South African vineyards and is dominant in the main vine growing areas of the Western Cape Province (Addison & Samways, 2000). Damage to vines is caused by moderate to high infestation of the pest. The mealybugs feed on vine roots, trunk, canes, leaves and fruit. The excretion of honeydew results in the growth of sooty mould on the leaves and the fruit. This reduces crop quality and yield (Walton *et al.*, 2004). *P. ficus* can also vector viral diseases (Engelbrecht & Kasdorf, 1990).

E. saccharina Walker is considered an important pest of sugarcane across Africa. The damage to the crop is as a result of the larvae boring into and feeding within the stems of the host plant (La Croix, 1992). Control of this pest is largely by predictive modelling and the application of chemical insecticides. There have been investigations into the use of parasitoids to control *E. saccharina*. However, there is a lack of positive results regarding effective parasitism and long term establishment of the parasitoids.

Only two *Lecanicillium lecanii* isolates caused low levels of mortality in *P. ficus* and three isolates resulted in mortalities of second and third instar larvae of *E.*

saccharina. Mean percentage mortality was low for all three isolates tested against *Eldana*, ranging from 11.6 - 21.4% over 15d. The use of entomopathogenic fungi to control both *P. ficus* and *E. saccharina* was not found in the literature and needs further study.

5.4   Future considerations and research needs

Having screened a number of entomopathogenic fungi against four agriculturally important pests found in RSA, it must be concluded that the susceptibility of these insects varied somewhat. This is not surprising as each fungus has its own optimum conditions under which infection takes place. With respect to mycoinsecticides, further improvements regarding mass production, stabilization, formulation and delivery rates are of critical importance (Faria & Wraight, 2001). The reliability of the fungi for pest control is still very dependent on the environmental conditions after application of the spores. The screening process is an important step in developing a biological control agent. However, unless formulations are developed that will reduce the reliance of entomopathogenic fungi on high levels of humidity, the potential of fungal biological control will not be realized. Researchers should target their activities towards (i) improved infection at low humidities and temperatures, (ii) improved dispersal, (iii) greater tolerance to insecticides so as to be used in an integrated control approach and (iv) an improved shelf life with lower costs. These points may be summarized as aiming to develop more reliable infection potential under unreliable conditions. From this study it was evident that two isolates, B1 and PPRI 6690, showed the most promise as future biological control agents against both *S. oryzae* and *T. vaporariorum*. With further consideration into the above-mentioned criterion, these two isolates could be formulated into commercial control products. Finally, infection of *P. ficus* and *E. saccharina* by entomopathogenic fungi was achieved in this study. The lack of literature on this subject seems to indicate that this may be a first report of such infection. Further research is needed in order to understand the potential implications of this result.

When comparing biological control to chemical control it is evident that biological control is at a clear disadvantage solely on the basis of immediate to short-term crop-production economics. Chemicals are able to control the pest population efficiently and effectively. However, an understanding of the limitations of biological control and the efficiency of chemical control, biological control has the potential to extend the market life of many chemicals if used in conjunction with them in a well-conceived IPM program.

5.5 References

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