FUNGAL PARASITISM OF CEREAL APHIDS IN SOUTH AFRICA

by

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[7] "…ask the animals,
and they will teach you,
or the birds of the air,
and they will tell you;
[8]or speak to the earth,
and it will teach you,
or let the fish of the sea
inform you.
[9]Which of all these does not know
that the hand of the LORD
has done this ?
[10]In his hand
is the life of every creature
and the breath of all mankind".

JOB 12: 7 – 10
New International Version of the
HOLY BIBLE

Dedicated to my wife Hesta, son Dylan, and daughter Melissa

ABSTRACT

The Russian wheat aphid, *Diuraphis noxia* is one of the most destructive exotic invaders of South Africa, capable of reducing individual wheat plant yields by up to 90%. Entomopathogenic fungi are important natural mortality factors associated with this aphid in its Eurasian endemic habitats as well as in the United States and Canada; their impact often exceeding that of predators and parasitoids.

The principal objectives of this study included (1) baseline characterization of the aphid-pathogenic flora associated with aphids from South Africa, with special reference to six common cereal aphids, (2) quantification of the comparative impact of the different fungal species on the cereal-aphid complex in three different wheat producing regions of South Africa, (3) field evaluation of the Hyphomycete *Beauveria bassiana* against *D. noxia* on resistant wheat, (4) screening of six fungicides for their potential usage in managing entomophthoralean epizootics within greenhouse rearings of the Russian wheat aphid, and (5) development and evaluation of a novel bioassay protocol for screening entomopathogenic Hyphomycetes against *D. noxia*.

A total of nine species of fungi known to infect and kill aphid hosts were collected, including the six entomophthoraleans, *Pandora neoaphidis*, *Conidiobolus thromboides*, *Conidiobolus obscurus*, *Entomophthora planchoniana*, *Conidiobolus coronatus* and *Neozygites fresenii,* and three Hyphomycetes, *Beauveria bassiana*, *Verticillium lecanii*, and *Paecilomyces farinosus.* The former four entomophthoraleans are considered first reports from this country. For the first time, morphological characteristics of these nine South African-collected species are visually depicted and techniques for their isolation and *in vitro* culture discussed.

Seven species of fungi were recorded from *D. noxia*, of which *P. neoaphidis* was the most important, causing up to 50% mortality during the late season under dryland conditions in the summer-rainfall region. Mycoses at epizootic levels, together with the large diversity of fungal species recorded from this host, indicated a high level of susceptibility to fungal infection. In contrast, infection of the oat aphid *Rhopalosiphum padi* remained < 5% despite favourable numbers of hosts and apparently suitable environmental conditions. This phenomenon strongly suggests some level of low susceptibility to fungal infection in this species.

Under irrigated conditions in KwaZulu-Natal, the rose-grain aphid *Metopolophium dirhodum* was the predominant aphid but remained below economical injury levels. Field surveys revealed that this aphid was effectively targeted by *P. neoaphidis* and *C. obscurus,* and findings suggest that in some areas of South Africa entomophthoralean fungi effectively suppress this aphid, negating the need for insecticide applications.

On average, *ca.* 61% control of *D. noxia* on resistant wheat was observed following an application of *B. bassiana* (5 x 10^{13} conidia per hectare) during the early flag-leaf stage of the wheat. Efficacy of *B. bassiana* applications on younger plants appeared to be influenced by the level of aphid activity, possibly explained by secondary pick-up of inoculum by *D. noxia*. These findings accentuate the importance of understanding the tritrophic relationship between host plant, pest and pathogen.

The fungicides copper oxychloride, mancozeb + oxadixyl, captab + metalaxyl, bittertanol, iprodione, and mancozeb at a rate of 0.1% active ingredient moderately to strongly inhibited *C. thromboides* vegetative growth (mean inhibition 81.1%). Mancozeb at concentrations of 10.0, 2.0, 1.25, 0.5, 0.08, and 0.016% was further evaluated *in vitro*. The fungus growth cut-off point, midway between 1.25 and 2.0% mancozeb, was calculated and a rate of 1.625% active ingredient per litre of water was used to decontaminate the fungus-infected *D. noxia* cultures.

A novel bioassay protocol was developed, employing live host plants for rearing aphids post inoculation. Using this design against *D. noxia*, an average LC_{50} estimate of 85 conidia per mm^2 for *B. bassiana* strain GHA was calculated. Control mortality was restricted to levels below 4%. The data indicated high precision due to an average coefficient of variation for slope of less than 20%, and an average chi-squared value of 5.46 ± 2.74 (n = 10 assays). The design will accommodate the use of cereal aphid species other than *D. noxia,* while live host plants will facilitate tritrophic studies on the effect of host-plant resistance on fungus-induced mortality of *D. noxia*.

Keywords: bioassay, control, entomopathogenic fungi, Entomophthorales, field, Hyphomycetes, oat aphid, rose-grain aphid, Russian wheat aphid, wheat.

PREFACE

This study represents the original work of the author and data presented in this thesis have not been submitted in any form to another university. Where the author referred to the work of others it has been duly acknowledged in the text.

Note that interim progress reports on fungal parasitism of the Russian wheat aphid were included in the 1996-2002 Annual Technology Reports of the Small Grain Institute of the South African Agricultural Research Council. However, the results presented in this thesis are based on complete and revised data sets. The University of Natal is acknowledged in all scientific publications compiled from the data presented in this thesis.

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CHAPTER 1

GENERAL INTRODUCTION

Aphids (Homoptera: Aphididae) are considered major pest insects in the agriculture of the temperate climatic zones (Vickerman & Wratten, 1979; Rabbinge *et al.*, 1981), and even considered by some as the most important agricultural arthropod pests (Minks & Harrewijn, 1988). Although aphids are more abundant in the Holarctic and Oriental regions than in the southern continents (Millar, 1994), some 136 aphid species are known from South Africa (Millar, 1990), of which more than 30 are of significance to cultivated crops (Annecke & Moran, 1982). Introduced species such as the black pine aphid, *Cinara cronartii* Tissot & Pepper (Van Rensburg, 1979, 1981), the blue-green lucerne aphid, *Acyrthosiphon kondoi* Shinji (Dürr, 1981), and the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Walters *et al.*, 1980), are considered serious pests in this country (Millar, 1990).

In South Africa, small grains, including wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), barley (*Hordeum vulgare* L.), and rye (*Secale cereale* L.) are prone to infestation by any of six aphid species (Annecke & Moran, 1982; Millar, 1990, 1994). These species are the greenbug, *Schizaphis graminum* (Rondani), the bird cherry-oat aphid, *Rhopalosiphum padi* (L.), the brown wheat ear aphid, *Sitobion avenae* (Fitch), the rose-grain aphid, *Metopolophium dirhodum* (Walker), the maize aphid, *Rhopalosiphum maidis* (Fitch), and *D. noxia* (Fig. 1.1).

1.1 Origin and pest status of the Russian wheat aphid in South Africa

Diuraphis noxia is believed to be endemic to the Iranian – Turkestanian range in central Asia (Kovalev *et al.*, 1991). This aphid was first described in the early 1900's when a major outbreak occurred in Moldova and southern Ukraine (Grossheim, 1914, translated by Poprawski *et al.*, 1992a). The aphid responsible for these outbreaks was initially identified as the barley aphid *Brachycolus korotnewi* Mordvilko (Mordvilko, 1901). This author later acknowledged (in Kurdjumov, 1913) that the aphid was in fact an independent species and proposed the new epithet *Brachycolus noxius* Mordvilko. This new description, however, was never formally published. With the permission of A.K. Mordvilko the name *B. noxius*

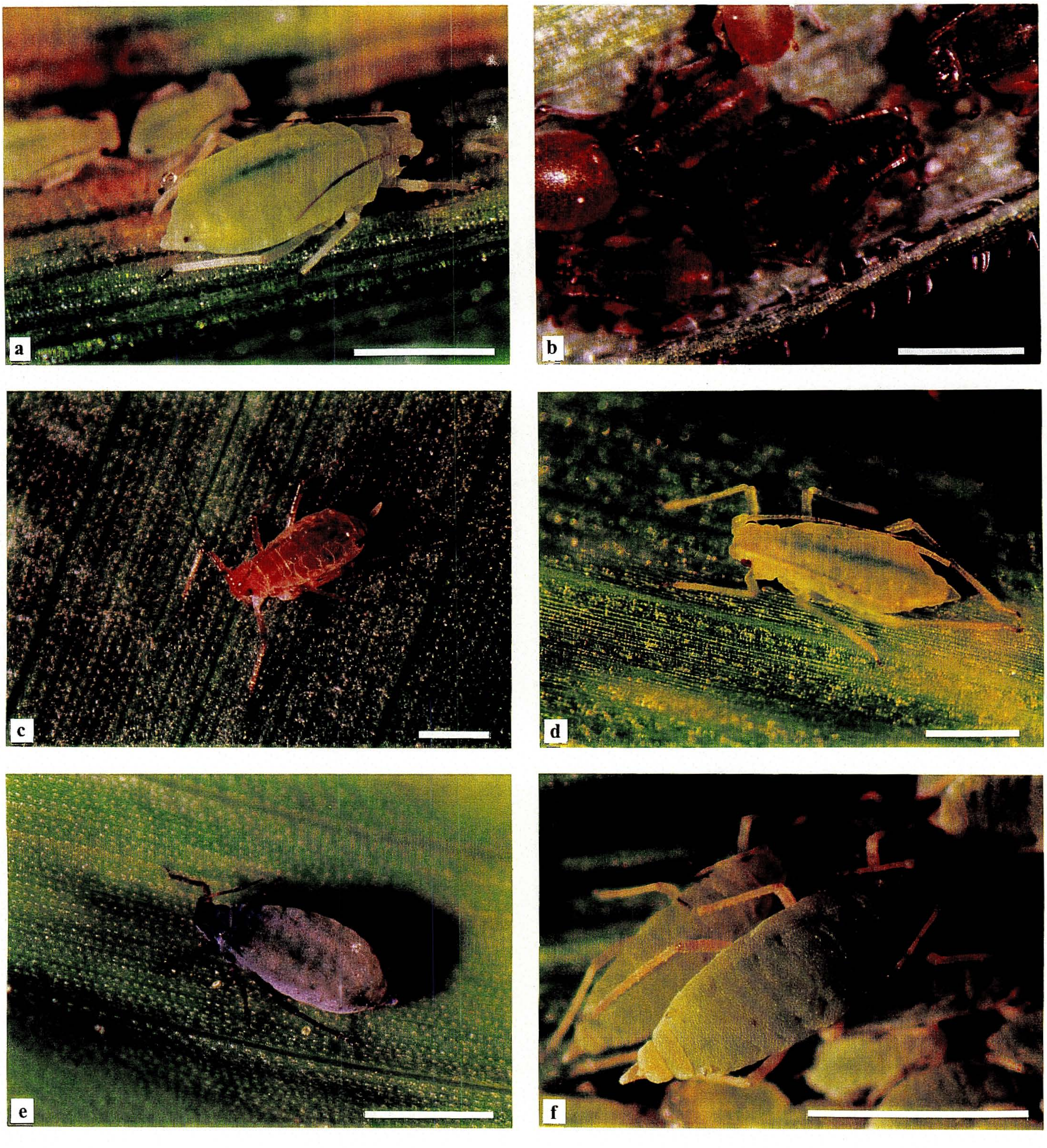


Figure 1.1. Cereal aphids. (a) Greenbug, *Schizaphis graminum*. (b) Oat aphid, *Rhopalosiphum padi*. (c) Brown wheat ear aphid, *Sitobion avenae*. (d) Rose-grain aphid, *Metopolophium dirhodum*. (e) Maize aphid, *Rhopalosiphum maidis*. (f) Russian wheat aphid, *Diuraphis noxia*. Bars = 1 mm.

was used in a new key published by Kurdjumov (1913) in which *B. noxius* was morphologically separated from other aphid species damaging small grains. Since then several synonyms and taxonomic shifts have appeared in the literature (see Eastop & Hille Ris Lambers, 1976; Dürr, 1983; Stoetzel, 1987; Hughes, 1988). The current authorship *Diuraphis noxia* (Kurdjumov), however, was determined by Kovalev *et al.* (1991) according to the provisions of the International Code of Zoological Nomenclature.

Diuraphis noxia was for the first time reported from South Africa in 1978 (Walters *et al.*, 1980), and has since become established as the principal pest of wheat cultivated under dryland conditions in the summer rainfall region (Du Toit, 1983; Aalbersberg *et al.*, 1988a). The potentially devastating effect of this aphid on wheat was reported by Du Toit & Walters (1984). Measuring individual plant yields, these authors recorded losses of up to 90%. Furthermore, *D. noxia* is seen as one of the main constraints to wheat production in Lesotho, where yields have decreased from *ca.* 1.8 ton ha^{-1} during the mid-seventies to current levels of *ca.* 0.8 ton ha^{-1} (Makhale *et al.*, 1999).

1.2 Feeding damage / symptoms associated with *D. noxia*

Cereal aphids, including *D. noxia*, are typically r-strategists (Dixon, 1985) and therefore characterised by high fecundity and short generation time (Dixon, 1987). These traits enable aphid populations to rapidly increase and disperse under field conditions (Robert, 1987). Generally, aphids cause crop damage by extracting plant sap (Wratten, 1975; Rabbinge *et al.*, 1981), by transmitting phyto-pathogenic viruses (Damsteegt & Hewings, 1988) or, a characteristic feature of *D. noxia* feeding damage (Fouché *et al.*, 1984; Krüger & Hewitt, 1984), by injecting toxic saliva as done by greenbug (Burton, 1986). Chloroplast destruction by *D. noxia* results in white or yellowish streaks developing along the entire length of the lamina (Du Toit, 1986). When streaking coincides with cold conditions, as often experienced in the Free State region of South Africa (Schulze, 1997), purpling of the streaks may occur due to the production of a pigment identified as cyanidine (Hewitt *et al.*, 1984). Moreover, inward curling of leaf edges, prostrate growth of young infested wheat plants and bent ears are characteristic feeding symptoms of this pest on susceptible wheat (Walters *et al.*, 1980).

Although several plant viruses are reportedly transmitted by *D. noxia* (Von Wechmar & Rybicki, 1981; Rybicki & Von Wechmar, 1984; Von Wechmar, 1984; Damsteegt *et al.*, 1988; Damsteegt & Hewings, 1988; Damsteegt *et al.*, 1992), economic damage caused by this aphid is primarily associated with toxemia rather than transmission of viruses (Hewitt *et al.*, 1984; Kriel *et al.*, 1984; Du Toit, 1986). Generally, *D. noxia* is a poor or non-vector of plant viruses (Burnett *et al.*, 1988; Summers *et al.*, 1990; Halbert *et al.*, 1992a, 1994).

1.3 Control programmes

The low thermal threshold for nymphal development is only 0.54°C (Aalbersberg *et al.*, 1987), enabling South African populations of *D. noxia* to rapidly increase in numbers during the early wheat season (September onwards). This coincides with reduced soil water content and stressed plant populations (Prinsloo *et al.*, 1995). These conditions and the ability of *D. noxia* to cause substantial damage at low infestation levels (Du Toit, 1984, 1986), initially required the use of insecticides, *i.e.,* combinations of parathion and a series of systemic organophosphates to control it (Walters *et al.*, 1980; Du Toit, 1983; Du Toit & Walters, 1984; Marasas *et al.*, 1997; Nel *et al.*, 1999). However, with more than 13 million Rands spent on insecticides for control of this aphid during 1986 in the eastern Free State alone (Du Toit, 1988), chemical control soon became too costly, especially in circumstances where the harsh climate under dryland conditions reduced the efficacy of foliar applied insecticides (Du Toit, 1992). Moreover, in low input agricultural systems, where financing, infrastructure, and technical know-how were not readily available, the use of insecticides was very limited and even prohibitive in terms of cost (Marasas *et al.*, 1997).

1.4 Integrated control of *D. noxia*

In view of the several disadvantages of chemical control, the Small Grain Institute (SGI) of the Agricultural Research Council (ARC), Bethlehem, South Africa, initiated an integrated-control programme based on host-plant resistance supported by natural enemies (Prinsloo *et al.*, 1995, 1997a). Host-plant resistance to the Russian wheat aphid was for the first time reported in South Africa in the late 1980's (Du Toit, 1987), with the subsequent release of the first commercial resistant wheat cultivar ('Tugela-DN'), in 1992 by ARC-SGI (Marasas, 1999). Currently, 12 wheat cultivars with resistance to *D. noxia* are commercially available in South Africa, of which five were developed at ARC-SGI (Prinsloo & Tolmay, 2002).

1.5 Rationale for including natural enemies in the integrated-control programme

Host-plant resistance can be explained by three functional categories, *i.e.,* antibiosis, antixenosis and tolerance, or combinations of these categories (Painter, 1951; Kogan & Ortman, 1978). The predominant source of resistance incorporated in all cultivars thus far released by the SGI is PI 137739 (DN1) (Marasas, 1999), conferring mainly antibiotic resistance (Du Toit, 1987, 1989a, 1989b). Antibiosis has been defined as the negative influence of the plant on the biology of the insect, *i.e.,* reduced fecundity, prolonged development of immature stages, reduced body size and/or failure to pupate or eclose (Smith, 1989). Following surveys conducted in the central and eastern Free State province, South Africa, it was projected that resistant cultivars released by ARC-SGI would, respectively, cover 46.7 and 54.5% of the area planted to wheat by the year 2000 (Marasas, 1999). These figures did not include other resistant cultivars marketed by private seed companies. Therefore, as the area planted with resistant cultivars is increasing annually, the host-plant-resistance selection pressure is likewise increasing. This selection pressure is one of three criteria mentioned by Puterka & Burton (1991) for the development of a resistance-breaking insect biotype. Moreover, compared with tolerance, the widespread use of a single antibiosis mechanism may force biotype differentiation (Gallun, 1972; Souza, 1998). This is a serious concern due to the high degree of biotypic diversity known to occur among certain populations of *D. noxia* (Puterka *et al.*, 1992, 1993; Robinson *et al.*, 1993). The only economical and environmentally sound approach in reducing host-plant-resistance selection pressure is through the indiscriminate mortality inflicted by natural enemies.

Recent surveys indicate that the Russian wheat aphid oversummers on several small grains including wheat, barley, oats, and rescue grass (*Bromus catharticus* Vahl), in the highlands of Lesotho (J.L. Hatting & G.J. Prinsloo, unpublished). Lesotho borders with the Free State province, a region in which approximately 45% of the South African wheat crop (2.1 million metric tons per annum) is produced as a winter crop (Purchase, 1997). On the other hand, wheat production in Lesotho occurs throughout the year, *i.e.,* as a summer crop in the highlands and as the most important winter crop in the lowlands and foothills (Massey *et al.*, 1990; Makhale *et al.*, 1999). Due to financial constraints, lack of equipment, and the often inaccessible terrain, chemical pest control is virtually non-existant in Lesotho. Clearly, this scenario together with the continuous production of wheat throughout the year, favours the use of natural enemies as a control option. The introduction of non-indigenous enemy species of *D. noxia* into Lesotho will not only benefit the local industry but will also reduce oversummering (January through April) populations of *D. noxia* in Lesotho, which might be a factor in reducing the level of infestation of the subsequent crop (Wraight *et al.*, 1993) in South Africa.

According to Marasas (1999) the maximum economical benefit of the integrated-control programme will only be realized once the natural enemy complex, together with resistant cultivars, become fully functional. By adding a "potential" biological control scenario to the base scenario (resistant cultivars alone), the net value at a 5% discount rate increased from 58.00 million Rands to 68.84 million Rands (Marasas, 1999). As also pointed out by Hajek (2002), successful classical biological control programmes can be extremely cost effective with cost benefits of up to 200:1. A trend of reduced pesticide usage soon became apparent after the release of the first resistant cultivar in 1992. According to a socio-economic impact analysis, the average area treated with foliar insecticides against *D. noxia* in the eastern Free State decreased from 85% in 1990 to 30% in 1997 and was projected to decrease to only 16% in 2000 (Marasas, 1999). Similarly, a projected 33% of the area in that region would have been planted with wheat grown from insecticide-treated seed in 2000 compared with 70% during the 1996 season (Marasas, 1999). Therefore, aphid mortality due to natural enemies will eventually eliminate the perception by farmers that chemical intervention is warranted because of immediate economical and environmental benefits.

The predominant insecticides used in the Free State include combinations of parathion with either demeton-S-methyl or monocrotophos, both systemic organophosphates (LD_{50} of 50 mg kg^{-1} rat body mass) (Marasas *et al.*, 1997; Nel *et al.*, 1999). These very toxic combinations are applied 1 – 3 times per season at a rate of *ca.* 1 litre per hectare (Marasas *et al.*, 1997). Parathion, sprayed for control of Russian wheat aphid during 1988 in Texas, was responsible for the death of 200 Canada geese due to anticholinesterase poisoning (Flickinger *et al.*, 1991). Previously, parathion poisoning of waterfowl was also reported by White *et al.*, (1982a, 1982b), following applications for control of *S. graminum* in northern Texas. South African wheat farmers (> 99% of farmers sampled) are generally in favour of biological control, but they have reservations about obtaining an effective biological-control component (Marasas *et al.*, 1997).

Although resistant wheat cultivars offer protection against *D. noxia*, such cultivars are not excluded from attack by any of the other five cereal-aphid species previously mentioned. A *D. noxia*-resistant genotype (PI 294994; variety 'IDO488'), conferring antibiotic/tolerant resistance, had no significant effect on *M. dirhodum, R. padi* and/or *S. avenae* populations during a two-year field trial in Moscow, Idaho (Schotzko & Bosque-Pérez, 2000). The trend in reduced chemical intervention may therefore lead to a resurgence of other important aphid pest species, such as *S. graminum* and *R. padi*, previously contained as a result of extensive Russian wheat aphid control initiatives (Rabe *et al.*, 1989). Prior to 1978, *S. graminum* was considered one of the most important pests of wheat produced under dryland conditions in the Free State province (Brown, 1969, 1974). An effective and diverse natural-enemy complex is therefore seen as an important means of restricting secondary pest species in the absence of insecticides.

1.6 Natural enemies associated with *D. noxia* in South Africa

The natural enemies of aphids include many species of predators, parasitoids and pathogens (Blackman, 1974; Cavalloro, 1983; Minks & Harrewijn, 1988), aiding in reducing the number of aphids within the crop, and/or regulating aphid populations between seasons (Vickerman & Wratten, 1979).

As mentioned above, *D. noxia* is endemic to Eurasia and although sporadic short-lived outbreaks have been reported from that region (*e.g.*, Tuatay & Remaudière, 1964; Kovalev *et al.*, 1991; Fernández *et al.*, 1992; Poprawski *et al.*, 1992a; Elmali, 1998) sustained economic damage over seasons is rare, suggesting that effective natural mortality factors play an important role in regulating Eurasian populations of *D. noxia*. However, studies conducted in South Africa revealed that natural enemies attacking *D. noxia* under dryland conditions in the summer rainfall region were unable to contain this aphid below injury levels (Aalbersberg, 1987; Aalbersberg *et al.*, 1988b; Prinsloo, 1990). Following is a list of natural enemies associated with *D. noxia* in Eurasia (Berest, 1980a, 1980b, 1985a, 1985b, 1987; Hughes, 1988; Pike & Allison, 1991; Aeschlimann & Hughes, 1992; Prinsloo & Neser, 1994; Elmali, 1998; Hopper *et al.*, 1998; Prokrym *et al.*, 1998). Species in bold represent a new association (*i.e.*, exotic *D. noxia* with indigenous enemy fauna) in South Africa (Aalbersberg *et al.*, 1984, 1988b; Prinsloo, 1990). Exotic species marked with asterisks were imported to South Africa although not necessarily released (see Table 1.1).

PREDATORS

Coleoptera (Coccinellidae)

- *Adalia bipunctata* (Linnaeus)*
- *Adalia fasciatopunctata revelierei* Mulsant
- *Coccinella septempunctata* Linnaeus*
- *Coccinella transversoguttata biinterrupta* (Mader)
- *Coccinella undecimpunctata* (Linnaeus)
- *Coccinula quatuordecimpustulata* (Linnaeus)
- *Exochomus quadripustulatus* (Goeze)
- *Hippodamia tredecimpunctata* (Linnaeus)
- *Hippodamia undecimnotata* (Schneider)
- ***Hippodamia variegata* (Goeze)**
- *Nephus bipunctatus* (Kugelann)
- *Oenopia conglobata* (Linnaeus)
- *Propylea quatuordecimpunctata* (Linnaeus)
- *Scymnus apetzi* Mulsant
- *Scymnus bivulnerus* Horn
- *Scymnus frontalis* (Fabricius)
- *Scymnus interruptus* (Goeze)
- *Scymnus marginalis* (Rossi)
- *Scymnus nigrinus* Kugelann
- *Scymnus rubromaculatus* (Goeze)
- *Subcoccinella* spp.
- *Thea vigintiduopunctata* (Linnaeus)

Coleoptera (Anthicidae)

- *Anthicus unicolor* Schmidt

Hemiptera (Anthocoridae)

- *Orius majusculus* (Reuter)
- *Orius niger* (Wolff)

Hemiptera (Nabidae)

- *Nabis ferus* (Linnaeus)
- *Nabis pseudoferus* Remane
- *Nabis punctatus* Costa

Diptera (Chamaemyiidae)

- *Leucopis argentata* Heeger
- *Leucopis atritarsis* Tanasijtshuk
- *Leucopis caucasica* Tanasijtshuk
- *Leucopis glyphinivora* Tanasijtshuk
- *Leucopis kerzhneri* Tanasijtshuk
- *Leucopis ninae* Tanasijtshuk*
- *Leucopis pallidolineata* Tanasijtshuk
- *Leucopis puncticornis* Meigen
- *Parochthiphila* sp.

Diptera (Syrphidae)

- *Chrysotoxum festivum* (Loew)
- *Episyrphus balteatus* (De Geer)
- *Megasyrphus annulipes* Zetterstedt
- *Megasyrphus* nr *annulipes* Zetterstedt
- *Meliscaeva auricollis* (Meigen)
- ***Metasyrphus corollae* (Fabricius)**
- *Metasyrphus luniger* (Meigen)
- *Metasyrphus nuba* (Wiedemann)
- *Scaeva pyrastri* (Linnaeus)
- *Sphaerophoria scripta* (Linnaeus)

Diptera (Cecidomyiidae)

- *Aphidoletes aphidimyza* (Rondani)

Diptera (Empididae)

- *Platypalpus pictitarsis* (Becker)

Neuroptera (Chrysopidae)

- *Anisochrysa flavifrons* Brauer
- *Chrysopa abbreviata* Curtis
- *Chrysopa formosa* Brauer
- *Chrysopa hungarica* Klapalek
- *Chrysopa phyllochroma* Wesmael
- *Chrysoperla carnea* (Stephens)

PARASITOIDS

Hymenoptera (Braconidae)

- *Adialytus* sp.
- ***Aphidius colemani* Viereck**
- *Aphidius ervi* Haliday
- *Aphidius matricariae* Haliday*
- *Aphidius picipes* (Nees)*
- *Aphidius rhopalosiphi* De Stephani*
- *Aphidius uzbekistanicus* Luzhetzki
- ***Diaeretiella rapae* (M'Intosh)**
- *Ephedrus persicae* Froggatt
- *Ephedrus plagiator* (Nees)
- *Lysiphlebus testaceipes* (Cresson)
- *Praon abjectum* (Haliday)
- *Praon gallicum* Stary
- *Praon necans* Mackauer
- *Praon volucre* (Haliday)

Hymenoptera (Aphelinidae)

- *Aphelinus albipodus* Hayat & Fatima
- ***Aphelinus asychis*** **Walker**
- *Aphelinus hordei* Kurdjumov*
- *Aphelinus toxopteraphidis* Kurdjumov
- *Aphelinus chaonia* Walker
- *Aphelinus varipes* (Foerster)

ENTOMOPATHOGENIC FUNGI (excluding findings reported in this thesis)

Entomophthorales (Zygomycotina)

- *Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller
- *Entomophthora planchoniana* Cornu
- *Neozygites fresenii* (Nowakowski) Remaudière & Keller
- *Pandora delphacis* (Hori) Humber
- *Pandora neoaphidis* (Remaudière & Hennebert) Humber
- *Zoophthora radicans* (Brefeld) Batko

Hyphomycetes (Deuteromycotina)

- *Beauveria bassiana* (Balsamo) Vuillemin
- *Paecilomyces fumosoroseus* (Wize) Brown & Smith

In addition, Aalbersberg *et al.*, (1984, 1988b), lists the following species as possible natural enemies of *D. noxia* in South Africa (*i.e.*, new association), although not recorded in association with *D. noxia* in Eurasia (see list above):

Coleoptera (Coccinellidae)

- *Cheilomenes lunata* (Fabricius)
- *Cheilomenes propinqua* (Mulsant)
- *Exochomus troberti concavus* Fürsch
- *Lioadalia flavomaculata* (De Geer)
- *Scymnus morelleti* Mulsant

Coleoptera (Carabidae)

- *Calosoma* sp.
- *Graphipterus atrimedius* Chaudoir

Acari (Anystidae)

- *Chaussieria* sp.

Acari (Erythracidae)

- *Erythraeus* sp.

In comparison with the predator and parasitoid-complex of *D. noxia* from Eurasia, less than 10% of these species are associated with this pest in South Africa. According to Aalbersberg *et al.* (1988b), the coccinellid *H. variegata* and the two braconids *A. colemani* and *D. rapae* were the most numerous and appeared to be the most clearly associated with *D. noxia* in South Africa. Conversely, *D. noxia*, collected from volunteer wheat and *Bromus* grass during 1987, was found parasitized exclusively by the aphelinid *A. asychis* (Prinsloo, 1990). *Aphelinus asychis*, although rarely found, was the only aphelinid trapped in wheat fields from 1983 through 1985 in the eastern Free State (Aalbersberg *et al.*, 1988b). As part of a classical biological control approach against *D. noxia*, several associated predator and parasitoid species were collected and imported to South Africa over the period 1980 through 2002 (Table 1.1).

From preliminary field trials, *A. hordei* shows excellent potential, capable of reducing caged *D. noxia* populations on resistant wheat by up to 50% compared with cages without wasps (Prinsloo *et al.*, 1995). Parasitism levels on susceptible wheat reached 83.3% following open field releases during 1993 at ARC-SGI, and up to 52.2 and 31.1% in 1994, in the Bethlehem and Ficksburg districts, respectively (Prinsloo, 1998). Establishment of *A. hordei* was recently confirmed by Prinsloo *et al.* (2002). The potential of *Aphelinus* spp. as biocontrol agents of Russian wheat aphid is further underscored by the findings of Yu (1992) and Farias (1995). According to Farias (1995), levels of parasitism of *D. noxia* by *Aphelinus* spp. in the Montpellier region, France, reached 55%, while parasitism by the braconid subfamily Aphidiinae was often comparatively lower when both wasp types were present.

Similarly, peak parasitism of *D. noxia* (*ca.* 16%) on irrigated spring wheat in southwestern Idaho, U.S., was mainly by the aphelinid *A. varipes* (Feng *et al.*, 1991a).

Table 1.1. Exotic natural enemies imported to South Africa from 1980 through 2002 for classical release against *D. noxia*

ENEMIES	ORIGIN	YEAR IMPORTED	RELEASED	ESTAB-LISHED	REFERENCE
PREDATORS **(Col: Coccinellidae)**					
Adalia bipunctata	UK/USA	1980	YES	YES	Du Toit (1983)
Hippodamia convergens[1]	USA	1980	YES	NO	Aalbersberg *et al.* (1984)
Coleomegilla maculata[1]	USA	1980	YES	NO	
Coccinella septempunctata	?	1980	NO	NO	
Hippodamia variegata[2]	Hungary	2002	YES	?	ARC-PPRI, unpublished data
(Dipt: Chamaemyiidae)					
Leucopis ninae (Three strains)	Pakistan, Iran, China	1994	YES	NO	Hatting (1995)
PARASITOIDS **(Hym: Braconidae)**					
Aphidius matricariae	Turkey	1988	YES	YES	Marasas *et al.* (1997)
Aphidius rhopalosiphi	Australia	1989	NO	NO	ARC-PPRI, unpublished data
Aphidius picipes	Australia	1990	NO	NO	
Diaeretiella rapae[2]	Turkey	2000	YES	?	
	Hungary	2002	YES	?	
(Hym: Aphelinidae)					
Aphelinus hordei	Ukraine	1991	YES	YES	Prinsloo & Neser (1994); Prinsloo (1998); Prinsloo *et al.* (2002).
Aphelinus asychis[2]	Hungary	2002	YES	?	ARC-PPRI, unpublished data

[1] Species initially imported for release against the black pine aphid, *C. cronartii* (ARC-Plant Protection Institute, unpublished data), hence their omission from list under 1.6.

[2] Exotic strains imported.

Entomopathogenic fungi have received very little attention as natural-mortality factors of *D. noxia* in South Africa. Only two observations of fungi (unidentified) attacking the cereal-aphid complex in the field have been reported from South Africa (Walters *et al.*, 1980; Von Wechmar *et al.*, 1991). However, surveys in the Eurasian endemic habitats of *D. noxia* and in North America have revealed a great diversity of entomopathogenic fungi infecting this aphid (see Chapter 2).

For the establishment of a successful biological- or integrated-control programme, a thorough knowledge of all natural-mortality factors at work in the field is required (Southwood, 1978; Van Huis & Meerman, 1997). The integrated-control programme presently being developed against *D. noxia* in South Africa is no exception and urgently called for additional research pertaining to entomopathogenic fungi.

Milner (1997) mentioned four strategies for exploiting fungal pathogens for aphid biological control:

(1) taking account of epizootics as part of natural control;
(2) introduction into a crop to induce an epizootic;
(3) classical biological control; and
(4) development of sprayable bioinsecticides.

Essentially, the study presented in this thesis is based on strategies (1) and (4). However, insight into the naturally occurring entomopathogenic fungal complex and impact thereof on cereal aphids in South Africa would provide clues as to the feasibility and possible implementation of the second and third strategies mentioned.

An adequate supply of healthy Russian wheat aphids on a continued basis is of crucial importance for host-plant resistance screening and as natural enemy laboratory hosts/prey. For this reason, the management of fungal epizootics within greenhouse rearings of the Russian wheat aphid, which are used for these purposes, was included (see Chapter 3). The subsequent Chapters (*i.e.,* Chapter 4 and 5) largely address the lack of information pertaining to the indigenous species composition and impact of entomopathogenic fungi associated with cereal aphids in South Africa. Chapter 6 reports on the efficacy of a commercial formulation of the hyphomycete, *B. bassiana*, in combination with antibiotic host-plant resistance against *D. noxia* under dryland field conditions. A novel bioassay methodology for screening entomopathogenic Hyphomycetes against cereal aphids is described in Chapter 7, followed by the evaluation of this protocol in Chapter 8.

CHAPTER 2

LITERATURE REVIEW

2.1 Microbial pathogens of insects with special reference to aphid hosts

Microbial pathogens of insects include noncellular, parasitic infectious entities (viruses), prokaryotic forms (bacteria), eukaryotic forms (fungi and protozoa), and although often larger and more complex than the abovementioned, nematodes (Tanada & Kaya, 1993). Viruses, protozoa, and bacteria primarily infect their hosts *per os*. However, access by bacteria is also gained through cuticular wounds (Humber, 1991). Entomopathogenic fungi are unique in their ability to directly penetrate the insect cuticle and do not require ingestion (Hajek & St. Leger, 1994). Although nematodes may also penetrate through the cuticle into the haemocoel (Tanada & Kaya, 1993), fungi are the only significant microbial pathogens of the piercing-sucking Homoptera, including aphids (Humber, 1991).

2.1.1 Taxonomy and identification of fungi known to infect aphids

Entomopathogenic fungi are found in the division Eumycota (Ainsworth, 1973) and include over 700 species (Hajek & St. Leger, 1994) of which at least 6% are known to infect aphid hosts (see 2.1.2 below).

All aphid-pathogenic fungi are classified within the subdivision Zygomycotina, class Zygomycetes, order Entomophthorales (with a single species reported from the order Mucorales), and in the Deuteromycotina, class Hyphomycetes. The last-mentioned subdivision is divided into classes based on the absence or presence of a fruiting body (*i.e.*, Hyphomycetes or Coelomycetes, respectively); no orders or families are recognized (Humber, 1991).

The most important species of Entomophthorales associated with aphids belong to the genera *Pandora*, *Entomophthora*, *Conidiobolus*, *Zoophthora*, and *Neozygites* (Latgé & Papierok, 1988; Humber, 1991; Milner, 1997). Although very few insect pathogenic Hyphomycetes seem to be specific to aphids, the genera *Verticillium*, *Beauveria*, *Metarhizium*, and *Paecilomyces* are considered the most important (Latgé & Papierok, 1988; Humber, 1991; Milner, 1997). The only hyphomycetous fungus regarded as a major aphid pathogen is *Verticillium lecanii* (Zimmermann) Viégas (Zimmermann, 1983; Humber, 1991; Milner, 1997). However, this fungus is normally ineffective under field conditions (Khalil *et* *al*., 1985) with natural epizootics observed only in humid, tropical or subtropical climates (Hall, 1981).

2.1.2 List of fungi known to infect aphid hosts

At least 44 species of entomopathogenic fungi have been reported from aphid hosts (Foster, 1975; Waterhouse & Brady, 1982; Samways, 1983; Samways & Grech, 1986; Nagalingam & Jayaraj, 1986; Hareendranath *et al*., 1987; Ozino *et al*., 1987; Latgé & Papierok, 1988; Santamarina *et al*., 1988; Mathai *et al*., 1990; Humber, 1991; Ben-Ze'ev, 1993; Milner, 1997; Humber, 1998; Rong & Grobbelaar, 1998). However, only seven of these species are considered major aphid pathogens (Humber, 1991).

The following list was compiled from the abovementioned literature. The seven major aphid-pathogenic species are printed in bold:

Subdivision: Zygomycotina
  Class: Zygomycetes
    Order: Entomophthorales
      Family: Entomophthoraceae
      Batkoa major (Thaxter) Humber
      Entomophthora chromaphidis Burger & Swain
      ***Entomophthora planchoniana* Cornu**
      Erynia erinacea (Ben-Ze'ev & Kenneth) Remaudière & Hennebert
      Pandora delphacis (Hori) Humber
      Pandora kondoiensis (Milner) Humber
      ***Pandora neoaphidis* (Remaudière & Hennebert) Humber**
      Pandora nouryi (Remaudière & Hennebert) Humber
      Tarichium atrospermum (Petch) Balazy
      Zoophthora anhuiensis (Li) Humber
      Zoophthora aphidis (Hoffman in Fresenius) Batko
      Zoophthora canadensis (MacLeod, Tyrrell & Soper) Remaudière & Hennebert
      Zoophthora occidentalis (Thaxter) Batko

Zoophthora orientalis Ben-Ze'ev & Kenneth
Zoophthora phalloides Batko
***Zoophthora radicans* (Brefeld) Batko**

Family: Ancylistaceae
Conidiobolus coronatus (Costantin) Batko
***Conidiobolus obscurus* (Hall & Dunn) Remaudière & Keller**
Conidiobolus osmodes Drechsler
***Conidiobolus thromboides* Drechsler**

Family: Neozygitaceae
***Neozygites fresenii* (Nowakowski) Remaudière & Keller**
Neozygites lageniformis (Thaxter) Remaudière & Keller
Neozygites lecanii (Zimmermann) Ben-Ze'ev & Kenneth
Thaxterosporium turbinatum (Kenneth) Kenneth & Ben-Ze'ev

Order: Mucorales
Family: Mucoraceae
Mucor circinelloides Van Tiegham

Subdivision: Deuteromycotina
Class: Hyphomycetes
Acremonium zeylanicum (Petch) Gams & Evans
Aspergillus clavatus Desmazières
Beauveria bassiana (Balsamo) Vuillemin
Cladosporium aphidis Thueman
Cladosporium cladosporioides (Fres.) de Vries
Cladosporium herbarum (Pers.) Link: Gray
Cladosporium oxysporum (Bert. & Curt.)
Fusarium oxysporum Schlecht.
Fusarium pallidoroseum (Cooke) Sacc.
Fusarium semitectum Berk.

Fusarium tricinctum (Corda) Sacc.
Hirsutella aphidis Petch
Hirsutella illustris Minter & Brady
Metarhizium anisopliae (Metsch.) Sorokin
Paecilomyces farinosus (Holm) Brown & Smith
Paecilomyces fumosoroseus (Wize) Brown & Smith
Penicillium minioluteum Dierckx
Verticillium fusisporum Gams
***Verticillium lecanii* (Zimmermann) Viégas**

As most observations on mycoses in cereal aphids concern entomophthoralean fungi (see section 2.2 below), additional information on their taxonomy and identification is included herein. A characteristic of most entomopathogenic Entomophthorales is the formation of conidia or ballistospores that are forcibly discharged from conidiophores emerging from hosts killed by these fungi. These conidia are released into the environment by any one of four mechanisms reviewed and explained in detail by Humber (1981) and Balazy (1993). In short, Balazy (1993) refers to these mechanisms as rounding off or papillar eversion (the simplest and most commonly observed mechanism), sporophore rocket, sporophore cannon, and passive detachment. As pointed out by Humber (1981), spore discharge is a major event in the life history of entomophthoralean fungi while also having taxonomic value, at least at the generic level.

Recent taxonomic literature pertaining to the Entomophthorales is extensive (see Remaudière & Keller, 1980; Humber, 1981, 1984, 1989, 1991, 1997; King & Humber, 1981; Ben Ze'ev & Kenneth, 1982; Waterhouse & Brady, 1982; Keller, 1987, 1991; McCoy *et al.*, 1988; Samson *et al.*, 1988; Balazy, 1993). A revised classification of the Entomophthorales was proposed by Humber (1989) in which six families are recognized: Entomophthoraceae, Completoriaceae, Ancylistaceae, Meristacraceae, Neozygitaceae, and Basidiobolaceae. In this revised classification, important taxonomic criteria used in the identification of the Entomophthorales include shape and size of primary conidia and papillae, karyology of primary conidia, conidiophores, and conidiogenous cells, mode of discharge, formation and morphology of rhizoids and/or cystidia, morphology of secondary conidia, and characteristics of resting spores.

A revision of the entomopathogenic Hyphomycetes was proposed by Carmichael *et al.* (1980) and Samson (1981). Samson *et al.* (1988) and Humber (1997) also keyed and illustrated the major Deuteromycotina and their ascomycetous sexual states (teleomorphs) associated with insects.

2.1.3 Media for isolation and *in vitro* culture of aphid-pathogenic fungi

For a comprehensive discussion on collecting, isolation, and culture of entomophthoralean fungi in general, readers are referred to Papierok & Hajek (1997). Some important aspects with regards to aphid-pathogenic fungi will be mentioned here (see also Chapter 4).

The major aphid-pathogenic fungi differ in their nutritional requirements and vary in the ease with which they can be grown on chemically defined media (Latgé & Papierok, 1988). Amongst aphid-pathogenic species within the same genus, these requirements may differ, an attribute of taxonomic importance. This was demonstrated by Humber & Feng (1991) in distinguishing *E. planchoniana* from *E. chromaphidis*; the latter fungus being easily isolated *in vitro* (Holdom, 1983) whereas similar media and techniques failed in yielding cultures of the former. In general, the basic elements required by entomopathogenic fungi include oxygen, water, an organic source of carbon and energy, inorganic or organic nitrogen, and macro- and micronutrients (Samson *et al.*, 1988). Compared with the Deuteromycotina, the entomopathogenic Zygomycotina require more complex growth media including an organic form of nitrogen (amino acids or proteins) and a carbon source such as dextrose or maltose (Samson *et al.*, 1988); sucrose is not metabolised by the Entomophthorales (Latgé, 1975).

Several major aphid-pathogenic entomophthoraleans have been isolated on media containing egg yolk. Feng & Johnson (1991) successfully cultured *P. neoaphidis* on SEMA (80% Sabouraud dextrose or maltose agar, 11.5% cow's milk, 11.5% egg yolk, and 0.1% Tween-80). Although basically similar, several other media containing egg yolk are reported in the literature (Balazy, 1993; Papierok & Hajek, 1997). *Conidiobolus* spp. and *Z. radicans* were also cultured on Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY), and were clearly less fastidious to culture than *P. neoaphidis* (Feng & Johnson, 1991). More sophisticated media have been developed for growing entomophthoralean protoplasts, but also for isolating species with high nutritional requirements. These media include medium M-199 (Morgan *et al.*, 1950), Grace's insect tissue culture medium (Grace, 1962), Schneider's medium (Schneider, 1964), MGM-443 (Mitsuhashi, 1982), and Glen (Beauvais & Latgé, 1988). Usually, Grace's medium is supplemented with 5% or 10% (v/v) heat-inactivated fetal bovine serum for optimal results, when isolating certain aphid-pathogenic fungi (Humber, 1991).

In addition to *V. lecanii*, hyphomycetous fungi with potential for use as aphid bioinsecticides include *B. bassiana*, *M. anisopliae*, *P. fumosoroseus*, and *P. farinosus* (Milner, 1997 and references therein). Compared with the Entomophthorales, these fungi are nonfastidious organisms and are easily grown on a wide range of conventional mycological media (Goettel & Inglis, 1997). One of the most commonly used mediums for isolation and culture of Hyphomycetes is Sabouraud dextrose agar supplemented with 1% yeast extract (SDAY) (Goettel & Inglis, 1997). Their amenability to mass production by both solid substrate fermentation and liquid fermentation render this group of fungi highly suitable for development as mycoinsecticides (Feng *et al.*, 1994; De la Torre & Cárdenas-Cota, 1996; Vega & McGuire, 1996; Goettel & Inglis, 1997; Jackson *et al.*, 1997; Milner, 1997).

2.2 Natural control of cereal aphids by entomopathogenic fungi

Dedryver (1983) defined three types of aphid mycoses depending on the ratio between the number of living aphids and aphids killed by the pathogen. The first type is an *epizootic* in which the number of mycosed aphids increases to eventually exceed the ratio of one mycosed to one live aphid. Destruction by mycoses happens more quickly than the aphid intrinsic rate of increase, leading to a total or nearly total disappearance of living aphids. The second type of mycosis is *enzootic* in which the number of mycosed aphids remains approximately proportional to the number of live aphids in both the logistic (period of exponential increase until the carrying capacity of the crop is reached) and subsequent decline (period of population decrease after carrying capacity is reached) phases (see Price, 1984 for a detailed discussion on insect population demography). This subsequently results in a certain balance between the aphids and their pathogenic agent. Although the pathogen does reduce the rate of increase, it only contributes to the decline of the aphid population and does not cause its extinction. Regarding Dedryver's third type, the level of mycosis remains insignificant and does not affect the development of the host population.

2.2.1 Western Eurasia

The first surveys aimed specifically at investigating the species complex and impact of entomopathogenic fungi on cereal aphids were reported in the early 1970's. Dean & Wilding (1971) observed three entomophthoralean species causing widespread epizootics within populations of *M. dirhodum* and *S. avenae* feeding on barley in England. During July of 1970, 40 – 76% of *M. dirhodum* and 34 – 80% of *S. avenae* were found infected by the entomophthoraleans *E. planchoniana*, *P. neoaphidis*, and *C. obscurus*. The same species were reportedly responsible for 60, 53, and 30% infection of *Metopolophium festucae* (Theobald), *M. dirhodum*, and *S. avenae*, respectively, on spring wheat during late June of 1971 (Dean & Wilding, 1973). Similarly, the three entomophthoralean species mentioned above also took part in natural control of cereal aphid populations in six out of eight years in France (Dedryver, 1983), and during 1983 in Finland (Papierok & Havukkala, 1986). Infection at epizootic level occurred during 1977 and 1980 in France and was attributed to *P. neoaphidis* (Dedryver, 1983). On the other hand, *E. planchoniana* was more abundant during the Finnish surveys. This was attributed to the exceptionally warm and dry summer of 1983 (Papierok & Havukkala, 1986), conditions believed to favour this pathogen (Remaudière, 1983; Papierok *et al.*, 1984; Papierok & Havukkala, 1986). From 1 942 specimens of *S. avenae* collected from cereals in several areas of Piemonte, Italy during June and July 1985-86, most were found infected by *E. planchoniana* (10.0-33.3%) and *P. neoaphidis* (3.3-32.4%) (Ozino *et al.*, 1988). Similarly, *E. planchoniana*, the predominant fungus, was identified from *ca.* 30% of cereal aphids (mainly *R. padi*) field-collected live and reared in the laboratory during a study in Denmark (Eilenberg *et al.*, 1996). Recently, a trial was conducted in Hungary to investigate the impact of plant density and natural-enemy exclosure on abundance of Russian wheat aphid, *D. noxia* and *R. padi* on barley. Results indicated *ca.* 35% and 50% mycosis of *R. padi* and *D. noxia*, respectively, by *P. neoaphidis*, the only fungal pathogen recorded (Basky & Hopper, 2000).

2.2.2 Eastern Eurasia

Under natural field conditions *P. neoaphidis* is the most commonly observed fungal pathogen of Russian wheat aphid in Eurasian endemic habitats of this pest. Reports of mycosis in Russian wheat aphid date back to 1910 during which an unidentified fungus killed up to 20% of *D. noxia* on barley in the Poltava area of the Ukraine (Kurdjumov, 1911).

Surveys in the Bitola region (Macedonia) during 1989 revealed up to 30% *P. neoaphidis*-infected *D. noxia* per tiller (Carl & Mohyuddin, 1989). *Pandora neoaphidis* was considered the most important natural mortality factor during the Macedonia survey and was also commonly found in the Chimkent region (southern Kazakhstan) during mid-1989 (Carl & Mohyuddin, 1989). However, no sign of fungal activity in the Bitola region was observed during the 1990 season (Carl & Izhevsky, 1990). This was mainly attributed to unfavourable macroclimatic conditions prevailing during that year. During foreign explorations for natural enemies associated with *D. noxia* throughout the whole of Eurasia (1988 – 1994), five species of entomopathogenic fungi were collected (Hopper *et al.*, 1998). Fungi collected included the three entomophthoraleans *P. neoaphidis, Z. radicans,* and *Conidiobolus* sp., and the two Hyphomycetes *B. bassiana* and *P. fumosoroseus.*

2.2.3 United States

The Russian wheat aphid was first reported from the United States in 1986 when it was found near Muleshoe, Texas (Stoetzel, 1987; Morrison *et al.*, 1988). Collection of a specimen by suction trap at Parma, Idaho, confirmed its spread to the Pacific Northwest during the following year (Halbert *et al.*, 1990, 1992b). Field surveys during 1986 – 1989 revealed a great diversity of entomopathogenic fungi attacking cereal aphids on irrigated grain crops in southwestern Idaho (Feng *et al.*, 1990a, 1991a). Ten species of Entomopathogenic fungi were recorded from *D. noxia, Macrosiphum euphorbiae* (Thomas), *M. dirhodum, R. maidis, R. padi, S. graminum,* and *S. avenae*, including the entomophthoraleans *P. neoaphidis, C. obscurus, C. coronatus, C. thromboides, E. chromaphidis, N. fresenii, Z. radicans, Z. occidentalis,* and the two Hyphomycetes *B. bassiana* and *V. lecanii*. *Pandora neoaphidis* was the most prevalent species annually in populations of *M. dirhodum* and *D. noxia* with *Conidiobolus* spp. secondary to *P. neoaphidis* in occurrence. *Entomophthora chromaphidis, N. fresenii, Z. radicans,* and *Z. occidentalis* were more sporadic and infected fewer aphid species. The two Hyphomycetes infected aphids in the field at very low levels compared with the Entomophthorales. Although epizootics occurred each summer, they usually developed too late to prevent aphid populations from damaging the crop. An exception occurred in Idaho during 1987 when *M. dirhodum* and *S. avenae* populations were effectively suppressed below economic levels. This was attributed to the unusually frequent rainfalls during late May and June of that year, enhancing the development of epizootics.

Metopolophium dirhodum experienced higher mortality from fungal infection than did *D. noxia* and *S. avenae*. The dominant fungus infecting *M. dirhodum* and *D. noxia* was *P. neoaphidis* whereas the three *Conidiobolus* spp. were more important on *S. avenae*. *Entomophthora chromaphidis* and two *Zoophthora* species sporadically infected cereal aphids. The two hyphomycetous fungi *B. bassiana* and *V. lecanii* were recorded from only 0.2 and 0.3%, respectively, of all field-collected cereal-aphid cadavers (Feng *et al.*, 1990a).

The niche typically occupied by aphid species on the wheat plant was hypothetically proposed by Feng *et al.* (1991a) as a major factor in determining the development and occurrence of mycoses. *Metopolophium dirhodum*, the most commonly infected aphid species, typically resides on the undersurface of the leaves (see also Annecke & Moran, 1982) thus being more exposed to fungal spores actively discharged or splashed from inocula overwintered on the soil surface. Moreover, in this microhabitat fungal inoculum is shaded from the lethal effects of UV radiation (see Leach, 1971 for a practical overview on the topic) while moderate temperatures and increased relative humidities favour survival, germination, and infection of the aphid host. In contrast, more xerophilic species such as *S. avenae* are exposed to an environment (upper leaves and developing ears) less suitable for germination of fungal spores and infection of aphid hosts. Latgé & Papierok (1988) also mentioned the unique feeding position of *M. dirhodum* compared with that of *S. avenae* as a possible reason for the difference in specificity of fungal species principally attacking these aphids. According to Latgé & Papierok (1988), *P. neoaphidis,* being less able to tolerate lower relative humidities than *E. planchoniana* (Dedryver, 1981; Remaudière *et al.*, 1981), more often attacked *M. dirhodum* whereas *S. avenae* was attacked by both *P. neoaphidis* and *E. planchoniana*. Such biotic and abiotic factors associated with epizootic development in the field were further analyzed by Feng *et al.* (1992b).

Although development of fungal infection in southwestern Idaho seemed to be better correlated with host density than with climatic factors, surveys in Montana and Colorado showed much higher correlations between environmental conditions and mycosis (Feng *et al.*, 1991b; Wraight *et al.*, 1993). During 1990 dryland (fall-sown winter wheat and spring-sown barley) and irrigated (late-sown spring wheat and barley) crops were surveyed in Montana (Feng *et al.*, 1991b). The dryland crops were heavily infested with Russian wheat aphid, but no fungal infection was recorded. Irrigated spring grains remained free of Russian wheat aphid infestation but were found infested with mainly *M. dirhodum* and *R. maidis* after early July. Both these aphid species were found infected with entomophthoralean fungi from late July through late August. *Pandora neoaphidis* was the most important fungus (45.9% of cadavers collected), followed by *C. obscurus, E. planchoniana*, and *Z. radicans*. The collection of *E. planchoniana* was a first record from cereal aphids in North America. Another entomophthoralean, *C. thromboides*, was isolated from *Diuraphis tritici* (Gillette) in 1998. However, infection of cereal aphids in irrigated crops in Montana remained low (< 10%) compared with that reported from southwestern Idaho (up to 90%) (Feng *et al.*, 1991a).

Feng *et al.* (1992a) discussed the population trends and biological aspects of cereal aphids, including the Russian wheat aphid, and their natural mortality factors on winter wheat in southwestern Idaho. Spatial patterns and sampling plans (numerical versus binomial) for four cereal-aphid species killed by entomopathogenic fungi and hymenopterous parasitoids in spring wheat (southwestern Idaho) were presented by Feng & Nowierski (1992). These authors found that the extent of aggregation was similar among *D. noxia* and *M. dirhodum* cadavers, which were generaly more aggregated than those killed by hymenopterous parasitoids. Similar research was conducted by Nowierski & Feng (1992) concerning spatial patterns and sampling plans for Russian wheat aphid and associated natural enemies.

Surveys to obtain baseline data on the identity and prevalence of the existing natural-enemy complex and relative impact on populations of Russian wheat aphid in Colorado were initiated during 1990 (Wraight *et al.*, 1993). In both irrigated and dryland fields of spring-planted wheat and barley surveyed, *D. noxia* was the most abundant aphid. The three entomophthoraleans *P. neoaphidis, E. chromaphidis,* and *C. obscurus* were recorded from irrigated fields although only *P. neoaphidis* reached epizootic levels (44% infection by mid-July). On the other hand, prevalence of fungal pathogens under dryland conditions did not exceed 2.5%, suggesting that pathogenic fungi exerted only limited pressure on *D. noxia* under dryland conditions in the semiarid American west.

During the following year only irrigated production systems were surveyed during which two methods of evaluation (dissection versus rearing) of parasitism and fungal infection were used (Wraight *et al.*, 1993). Although the two methods produced similar estimates of parasitism, the rearing method detected *ca.* 25% more fungal infection than the dissection method. However, pooled estimates from the two methods were considered more reliable due to the difficulty in determining early stages of fungal infection through dissection (underestimation) and favourable holding conditions inducing fungal spores to germinate and penetrate the host (overestimation). During these surveys the Russian wheat aphid population never exceeded 0.5 aphids per tiller in fall-planted wheat, with no pathogen activity detected. However, parasitism of *D. noxia* peaked during mid-June at 76%. In contrast, parasitoid prevalence remained low (not exceeding 37%) in spring-planted grain. *Pandora neoaphidis* was the only fungus detected, *i.e.*, within populations of *M. dirhodum* (8% infection) and *D. noxia* (2.3% infection). However, infection of *D. noxia* on late-planted wheat increased from 7.3% to peak at nearly 80%, causing a decline of 81% in the peak density of 20.1 aphids per tiller recorded during mid-July. Once again, the epizootic-causing fungus was *P. neoaphidis*, with *E. chromaphidis* and *C. thromboides* averaging only 7% infection.

Wraight *et al.* (1993) concluded by recognizing the need for research addressing four fundamental issues: (1) Control of early-season aphid populations requires disease development under cool weather conditions. Therefore, screening and selection of pathogen strains with below-normal temperature optima may alleviate this problem. (2) Delivery of fungal inoculum into *D. noxia* colonies developing within tightly rolled leaves of infested tillers should be addressed. Three possible techniques were proposed, application of dry-formulated or fresh fungal mycelium or conidia, release of infected aphids, or release of fungus-inoculated hymenopteran parasitoids (see Poprawski *et al.*, 1992b). (3) A third problem involves the dependence of entomopathogenic fungi on high moisture conditions for sporogenesis, spore germination, and infection of the host. Irrigated cropping affords the opportunity to artificially manipulate moisture conditions to favour disease development. Optimal irrigation schedules should therefore be developed which may aid in the transmission of aphid-pathogenic fungi. (4) The potential importance of entomopathogenic fungi as antagonists of parasitoids (see El-Maghraby *et al.*, 1988; Brooks, 1993) and predators underscores the need for a better understanding of the host-pathogen-parasitoid-predator interaction. On the other hand, this interaction may be inconsequential (Milner *et al.*, 1984; Fransen & Van Lenteren, 1994), or even mutually benefical (Mesquita *et al.*, 1997). Biological control strategies must therefore be developed to enhance the integrated operation of these major groups of aphid antagonists.

2.2.4 Canada

The arrival of the Russian wheat aphid in southern Alberta, Canada, in 1988 (Jones *et al.*, 1989) prompted surveys to characterize the native or previously established natural-enemy complex active in the region. Surveys during 1989 and 1990 revealed two entomophthoralean species, *C. obscurus* and *E. chromaphidis*, attacking *R. padi* and several unidentified aphids (Doane *et al.*, 1992). In 1989, Goettel *et al.* (1990) surveyed cereal-aphid populations in southern Alberta and reported > 90% mycosis in mixed populations of *S. graminum* and *R. maidis* occurring during late August; epizootics were caused by (unspecified) Entomophthorales. Above-average rainfall during August of that year (78.4mm versus 41.4mm average) may have provided conditions necessary for epizootic development (Olfert *et al.*, 2001). Two entomophthoraleans, *P. neoaphidis* and *C. obscurus*, were identified from *D. noxia* during September of that year although at very low levels (Goettel *et al.*, 1990). Follow-up surveys in southern Saskatchewan during 1990 yielded only a few mycosed *S. avenae* infected by *C. obscurus* (Goettel & Erlandson, 1991). However, during 1992, *D. noxia* infestations reached the ecomomic threshold in many spring-seeded cereal fields. Epizootics (due to *P. neoaphidis*) only developed during late August in 75% of 48 fields sampled. Goettel *et al.* (1993) concluded that the importation of exotic fungi with ability to cause epizootics under cool conditions, *i.e.*, after September, would be most beneficial in protecting Canadian winter wheat crops.

2.2.5 Africa

The species complex and impact of entomopathogenic fungi on cereal aphid populations on the African mainland remains largely unexplored. The Russian wheat aphid is known from at least eight African countries including Morocco, Algeria, Libya, Egypt, Ethiopia, Kenya, South Africa and Lesotho (Walters *et al.*, 1980; Haile, 1981; Kriel, 1984; Attai & El-Kady, 1988; Hopper *et al.*, 1998; Makhale *et al.*, 1999; Wanjama & Arama, 1999).

Diuraphis noxia was first reported from Ethiopia in 1972 and had spread to all barley and wheat producing regions by 1976. Following these discoveries, *D. noxia* soon became the principal pest of cereals in the highlands of Ethiopia (Haile, 1981; Haile & Megenasa, 1987). During 1988 – 1990 widespread epizootics were observed among populations of *D. noxia* in barley and wheat in the Ethiopian highlands of Tigrai and Welo. The etiological agent responsible for these epizootics (> 60% mortality) was identified by Dr. T.J. Poprawski as the entomophthoralean *N. fresenii* (Poprawski & Wraight, 1998). As previously mentioned, only two observations of fungi attacking the cereal-aphid complex in the field have been reported from South Africa. Walters *et al.* (1980) reported on an unidentified fungus attacking Russian wheat aphid during the later part of the wheat growth season, *i.e.*, during warm, moist summer conditions, in the Free State province. No further detail or description of mycosed aphids were provided. A very unusual association of aphid-infecting viruses with entomophthoralean fungi (unidentified) was later reported by Laubscher *et al.* (1990) and Von Wechmar *et al.* (1991). According to these authors aphid lethal paralysis virus (ALPV) and *Rhopalosiphum padi* virus (RhPV) occur naturally in South African populations of *R. padi, D. noxia, S. avenae, S. graminum*, and *M. dirhodum.* However, the presence of ALPV and RhPV was also detected in dissected aphids infected by unidentified entomophthoralean fungi as well as in fungal hyphae enveloping dead aphids (ALPV only; Laubscher *et al.*, 1990). Although also found in wheat and barley (Von Wechmar, 1984), RhPV was characterised essentially as an aphid pathogen by D'arcy *et al.* (1981).

During 1985 *D. noxia* was also observed attacking wheat and barley in the Beni-Suef Province in Egypt and has subsequently spread also to other cereal producing regions of that country (Attai & El-Kady, 1988). However, no information regarding fungal parasitism of *D. noxia* was reported. Recently, surveys at the National Plant Breeding Research Center at Njoro, Kenya, revealed two entomophthoralean species, *i.e.*, *P. neoaphidis* and *E. planchoniana,* infecting *M. dirhodum* (Wanjama & Arama, 1999).

2.2.6 Current review

Surveys in many parts of the world have underscored the importance of entomopathogenic fungi in regulating cereal aphid populations. As pointed out by Feng (1990) the impact of these fungi on aphid populations often exceeds that of predators and parasitoids. This has been the case during some years in Europe (Dean & Wilding, 1971, 1973; Rautapää, 1976; Rabbinge *et al.*, 1979; Chambers *et al.*, 1983; Dedryver, 1983; Basky & Hopper, 2000), South America (Lázzari, 1985), and North America (Feng *et al.*, 1991a; Wraight *et al.*, 1993).

Clearly, however, there is a paucity of information regarding the species complex and impact of entomopathogenic fungi associated with cereal aphids from the African continent. In this regard, the study presented herein was aimed specifically at the South African scenario, providing baseline information on the use of these fungi against the cereal-aphid complex.

CHAPTER 3

MANAGING THE ENTOMOPATHOGENIC FUNGUS *Conidiobolus thromboides* IN RUSSIAN WHEAT APHID REARINGS

3.1 Introduction

The Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), is the most serious pest of wheat in the summer-rainfall region since it entered South Africa in 1978 (Walters *et al.*, 1980). As an alternative to chemical insecticides, an integrated-control programme for Russian wheat aphid, using resistant cultivars in combination with parasitoids, predators, and entomopathogenic fungi, is being developed at the Small Grain Institute (SGI) of the Agricultural Research Council (ARC) (Prinsloo *et al.*, 1995, 1997a).

Genetically resistant wheat cultivars are seen as an ideal management option for Russian wheat aphid control as they offer both economic and effective control. The first Russian wheat aphid resistant cultivar ('Tugela DN'), bred by ARC-SGI, was released for commercial usage in South Africa in 1992 (Marasas, 1999). Currently, 12 *D. noxia*-resistant wheat cultivars are commercially available in South Africa, of which five were developed at ARC-SGI (Prinsloo & Tolmay, 2002).

The entomopathogenic fungus *Conidiobolus thromboides* Drechsler (Zygomycetes: Entomophthorales) has been isolated in North America, Asia, Australia, and Europe from soil detritus and often from dead insects, mostly aphids (Balazy, 1993). This pathogen can kill its aphid host within one day after penetration of the haemocoel (Latgé *et al.*, 1980) with death probably resulting from the production of mycotoxins (Claydon & Grove, 1978). Entomopathogenic strains of this major aphid pathogen (Humber, 1991; see also Chapter 2) have been widely studied as potential biological control agents of aphids (Humber *et al.*, 1977; Latgé & Papierok, 1988). In this regard, *C. thromboides* is a beneficial microorganism. However, insect mass rearing in the greenhouse and insectary is often challenged by both biotic and abiotic factors governing the success of production (King & Leppla, 1984). Laboratory populations are kept at constant environments with stable abiotic factors (*i.e.*, temperature, light, wind and humidity). The most common microbial contaminants encountered under such conditions are fungi, followed by bacteria, viruses, protozoans and nematodes (Van Lenteren, 1991). A *C. thromboides* infection was detected within Russian wheat aphid cultures maintained in support of the host-plant-resistance and natural-enemy programmes of ARC-SGI. Despite scrupulous sanitation, reduced humidity levels, and a regimen of fluctuating temperatures, epizootics continued to develop. The continuation of several research programmes aimed at developing new resistant cultivars and the introduction/evaluation of exotic natural enemies, including entomopathogenic fungi, required better management and/or elimination of fungal epizootics occurring in the greenhouse. The implementation of chemical control of the fungus was thus considered and subsequently *in vitro* tests were conducted in this regard.

The objectives of this laboratory study were (1) to evaluate chemical fungicides for their efficacy in inhibiting (fungistasis) or suppressing (fungicidal) vegetative growth and sporulation of *C. thromboides* and (2) to determine the concentration at which an effective, but inexpensive, fungicide, in combination with sanitary measures, should be applied for control of the fungus in the greenhouse.

3.2 Material and methods

The common, trade and chemical names, concentration of active ingredient, and supplier of the fungicides tested in this study are listed in Table 3.1. All fungicides tested are registered for use in South Africa (Nel *et al.*, 1999). The fungicides were tested for their effects on *C. thromboides* at the rate of 0.1% active ingredient (0.1g active ingredient per 100 ml of culture medium). Both the stock culture and test cultures of the test fungus were grown on a wheat-grain-extract (WGE) medium (Srinivasan *et al.*, 1964). The isolate of *C. thromboides* used in the study originated from an infected Russian wheat aphid collected from a greenhouse at ARC-SGI (strain PPRI 6014, see Chapter 4). The stock culture was initiated by placing a plug (*ca.* 10 mm x 10 mm) of the sporulating original culture of *C. thromboides* in the centre of a 9-cm Petri dish containing the growth medium. Inoculation procedures were carried out on a laminar flow bench. The inoculated dish, with lid replaced, was incubated for 7 days (d) at 20°C under a photophase of 16:8 (Light:Dark) (L:D) h. The required quantity of each fungicide was added to 100 ml of sterilized medium cooled down to 65°C in a water bath. Mixing of medium and fungicides took place on a magnetic stirrer for *ca.* 5 min. Twenty-five ml of the medium treated with each fungicide were then poured into 9-cm Petri dishes. Fungal plugs (4 mm x 4 mm x 2 mm) consisting only of mycelia were

Table 3.1. Details of the fungicides tested to control *C. thromboides* infecting laboratory cultures of Russian wheat aphid in the greenhouse.

Common name (Trade name)	**% active ingredient**	**Chemical name**	**Supplier**
Copper oxychloride (Cupravit)	85	Basic cupric chloride	Bayer (Pty) Ltd South Africa
Mancozeb	56	Manganese ethylenebisdithiocarbamate with zinc ion	Sandoz Products Ltd South Africa
+ oxadixyl (Sandofan)	+ 8	+ 2-Methoxy-N-(2-oxo-1,3-oxazolidin-3yl)acet-2',6'-xylidide	
Captab	35	N-(trichloromethylthio)cyclohex-4-one-1,2-dicarboximide; 1,2,3,6-tetrahydro-N-(trichloromethylthio)phthalimide	Ciba-Geigy (Pty) Ltd South Africa
+ metalaxyl (Apron C)	+ 35	+ Methyl N-(2-methoxyacetyl)-N-(2,6-xylyl)-DL-alininate	
Bitertanol (Baycor)	30	β-([1,1'-biphenyl]-4-yloxy)-α-(1,1-dimethylethyl)-1H-1,2,4-triazole-1-ethanol	Bayer (Pty) Ltd South Africa
Iprodione (Rovral Dip)	50	3-(3,5-Dichlorophenyl)-N-(1-methylethyl)2,4-dioxo-1-imidazolidinecarboxamide	Rhone-Poulenc Agrichem SA (Pty) Ltd South Africa
Mancozeb (Sancozeb)	80	Manganese ethylenebisdithiocarbamate with zinc ion	Sanachem South Africa

excised from the outer perimeter of the stock culture and one plug then transferred (on a laminar flow bench) to the center of each Petri dish. Four replicated dishes of each treatment and control (nontreated medium) were maintained inside a laboratory incubator at 20°C and photoperiod of 16:8 (L:D) h. The dishes of each treatment and control were sealed with parafilm to minimize volatile products or by-products from escaping which could influence the fungal growth on other treatment or control dishes.

Vegetative (mycelial) radial growth, exclusive of the radius of the inoculum, was measured daily for 7 d. Measurements were taken with a millimetric ruler along the same pre-marked radial line on each occasion. Daily values were cumulated; the replicated values recorded for each treatment and the control were averaged (± Standard Error of Means). The 7-d cumulative growth data were analyzed by one-way analysis of variance using the SYSTAT package version 5.2 (Wilkinson *et al.*, 1992), and means were separated using the Tukey Honestly Significant Difference (HSD) test. The 7-d Abbott-corrected (Abbott, 1925) percentages of inhibition were calculated as: [(growth on nontreated medium - growth on fungicide-treated medium) / (growth on nontreated medium)] x 100. The lid of each Petri dish was inspected on day 7 for the presence of ballistospores (forcibly discharged entomophthoralean spores) as indicative of sporulation. Sporulation was not quantified but estimated for intensity, and classified as either absent, sparse, dense, or profuse. Where no sporulation was observed after 7 d on fungicide-treated medium, the fungus was assessed for viability. Plugs of the mycelium (*ca.* 5 mm x 5 mm) were excised from each replicated Petri dish and transferred to fresh, nontreated WGE medium (4 plugs were introduced into each of 3 replicated dishes). The dishes were incubated for 5 d at 20°C and photoperiod of 16:8 (L:D) h. The plugs were then examined for any sign of regrowth radially extending from each initial plug. The Petri dish lid directly above each plug was checked for the presence of ballistospores. Regrowth and sporulation were indicative of a fungistatic rather than fungicidal effect of a given fungicide on *C. thromboides*.

To identify fungal growth and sporulation cut-off points, one fungicide strongly inhibiting *C. thromboides* (Sancozeb; see Fig. 3.1) and widely available at low cost, was further tested *in vitro* at concentrations of 10.0, 2.0, 1.25, 0.5, 0.08, and 0.016% active ingredient (mancozeb) per 100 ml medium for its effects on the fungus. Methods and analyses used were as described above.

In an attempt to decontaminate the rearing facilities, every greenhouse used for this purpose was washed with water and then fumigated with potassium permanganate ($KMnO_4$) added to formaldehyde (4.3 litres formaldehyde plus 1.8 kg $KMnO_4$ for treatment of 283 cubic metres). All cages (50cm^3 steel frames covered with nylon sheer) were washed with the foaming detergent/sanitiser Asepto Z (a chlorinated alkaline with a concentration of 1-1.5 %; Klenzade SA (Pty) Ltd, South Africa).

Actively-feeding field aphids were collected to start new colonies on a small scale prior to moving the aphids into the larger insectaries inside the treated greenhouses. As soon as Russian wheat aphids were introduced into the cages, the plants, aphids, and soil surface were sprayed with Sancozeb at 20 g per litre of water using a hand-held sprayer. At each aphid cycle (*ca.* 10 days), the cages were again washed with Asepto Z and the new plants, aphids, and soil surface sprayed with Sancozeb. As a control, one greenhouse and the cages housing cultures of parasitoids were treated with all of the above except the fungicide treatment because of possible negative effects of the latter on the parasitoid wasps.

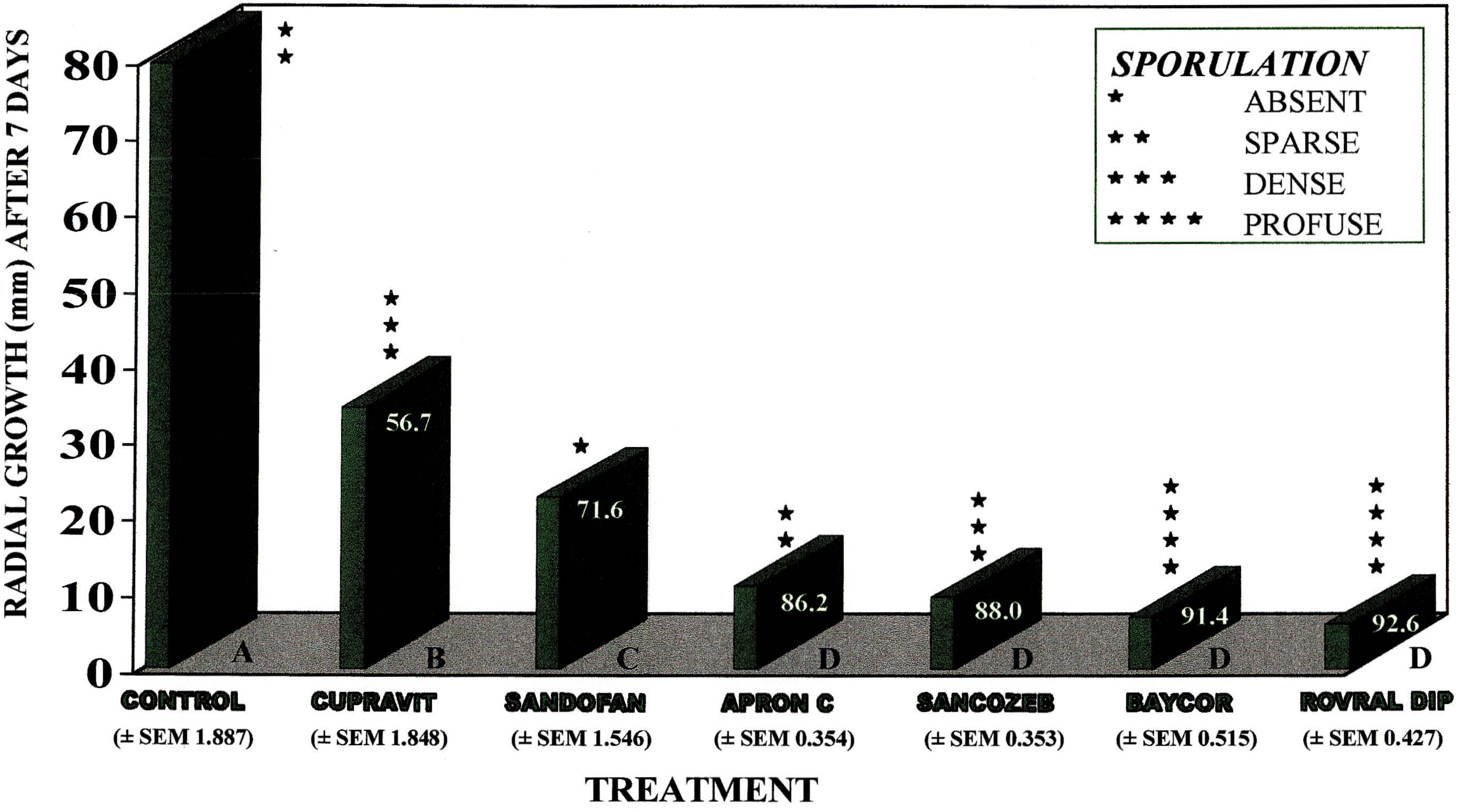
3.3 Results

The effects of fungicides at 0.1% rate of active ingredient on the development of *C. thromboides* on solid medium are shown in Figure 3.1. All of the fungicides tested had some degree of fungistasis to *C. thromboides*, but no truly fungicidal activity was observed with any of the fungicides. There were significant differences in mycelial growth among the treatments ($F = 489.1$; df = 6, 21; $P < 0.001$).

Of the fungicides tested, both Sandofan and Cupravit moderately inhibited *C. thromboides*, reducing mycelial growth by 71.6 and 56.7%, respectively. After 7 d of development, sporulation intensity in the Cupravit treatment was high. No spores were produced on Sandofan-treated medium. However, mycelial growth and sparse sporulation occurred after 5 days of subculturing *C. thromboides* on Sandofan-free medium. Apron C, Sancozeb, Baycor and Rovral Dip strongly inhibited growth of the fungus over the 7 d. The 7-d percentage of inhibition of vegetative growth by these four fungicides was > 86%.

Spore production at 7 days was sparse on the medium treated with Apron C. However, as compared with the control, Sancozeb, Baycor, and Rovral Dip enhanced the formation of spores. Because of its strong inhibitory effect on *C. thromboides* vegetative growth (Fig. 3.1) and low costs, Sancozeb was further evaluated at six different concentrations of active

Figure 3.1. Effect of 0.1% active ingredient of fungicides on radial growth and sporulation of *C. thromboides*



LETTERS NEXT TO BARS REPRESENT SEPARATION OF MEANS OF RADIAL GROWTH (TUKEY HSD test). VALUES IN BARS REPRESENT 7-d CONTROL- CORRECTED PERCENTAGES OF INHIBITION OF RADIAL GROWTH.

ingredient. The effect of these concentrations on fungal growth over 7 d is shown in Figure 3.2.

There were significant differences in mycelial growth among the treatments ($F = 3.666$; df = 6.21; $P < 0.001$). Mancozeb at 2.0 and 10.0% was fungicidal and completely inhibited growth and sporulation of *C. thromboides*. Mancozeb was moderately inhibitory at 0.016% and at 0.08 and 0.5% highly inhibitory of mycelial growth; however, sporulation was dense at these three concentrations. Very limited growth and no sporulation occurred when mancozeb was used at a concentration of 1.25%. The fungus growth cut-off point, midway between 1.25 and 2.0% mancozeb, was calculated as [1.25 + 2.0]/2 and a rate of 1.625% mancozeb (20g Sancozeb) per litre of water was used to treat fungus-contaminated greenhouses and rearing cages in the validation study.

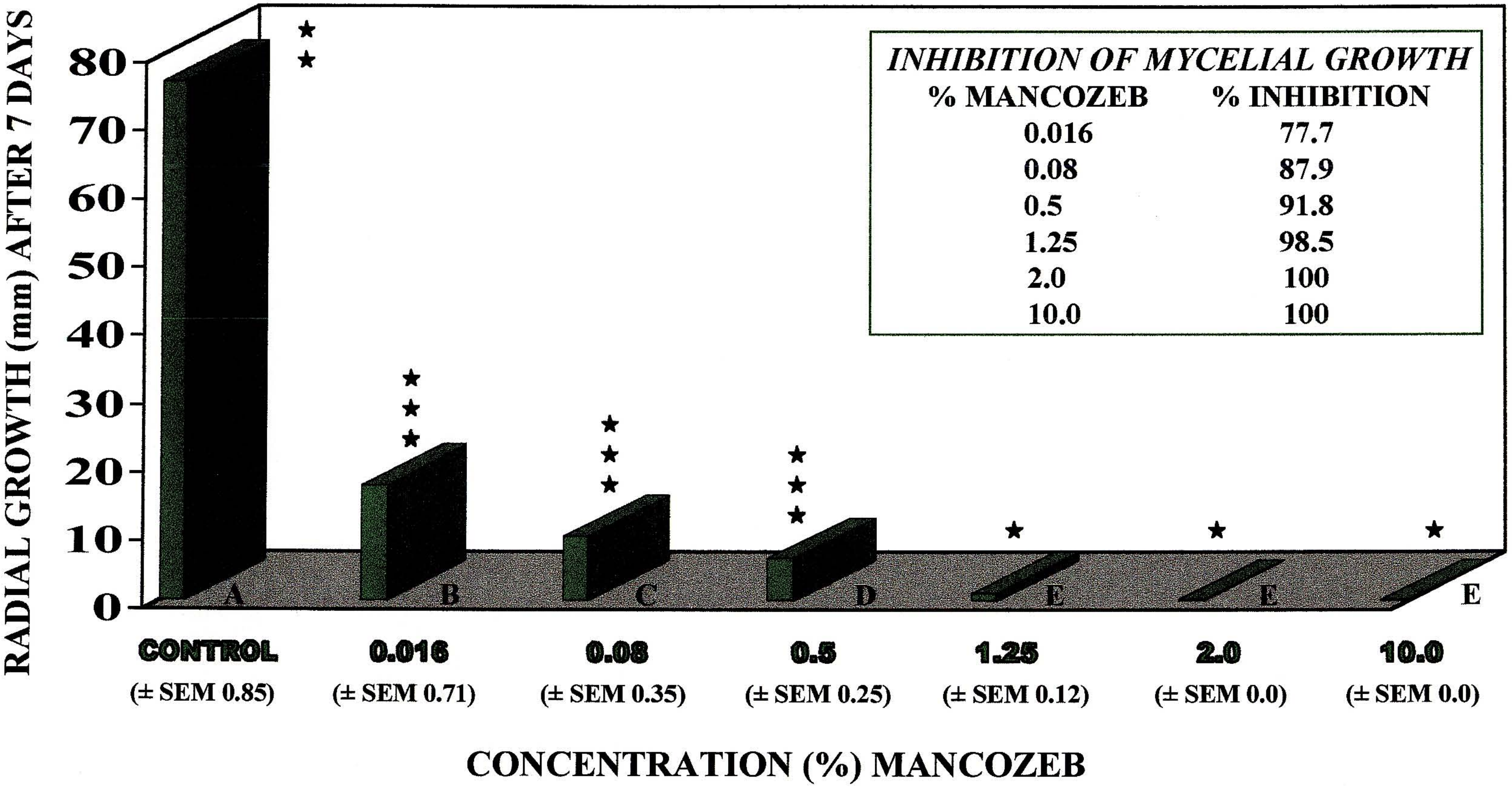
No resurgence of the fungal problem occurred for 2 months after Sancozeb and sanitary measures were used to treat the *C. thromboides*-contaminated Russian wheat aphid cultures. However, the fungal problem resurged in the greenhouse housing the parasitoid cultures when no Sancozeb was applied. Sancozeb had no acute or sublethal effects on *D. noxia* (data not shown).

3.4 Discussion

These laboratory findings were generally consistent with previously published observations made *in vitro*. The effect *in vitro* of copper oxychloride and related copper-based inorganic compounds on entomopathogenic fungi has been variable. For example, Soper *et al.* (1974) found copper hydroxide completely inhibited four species of Entomophthorales. Majchrowicz & Poprawski (1993) reported moderate-to-complete inhibitory action of copper oxychloride on *C. thromboides, Conidiobolus coronatus* (Costantin) Batko (Zygomycetes: Entomophthorales), and several species of entomopathogenic Hyphomycetes.

Fungicides such as bitertanol, based on triazoles, have been little studied with regard to their effects on entomopathogenic fungi. Regarding the Hyphomycetes, tests by Hassan *et al.* (1991) found that 0.0925% bitertanol was highly toxic to *Verticillium lecanii* (Zimmerman) Viégas, but harmless to *Beauveria bassiana* (Balsamo) Vuillemin. Azoles were moderately harmful to harmful to *Beauveria brongniartii* (Saccardo) Petch (Coremans-Pelseneer *et al.*, 1991; Coremans-Pelseneer, 1994). Six species of hyphomycetous entomopathogenic fungi

Figure 3.2. Effect of different concentrations of mancozeb on radial growth and sporulation of *C. thromboides*



INHIBITION OF MYCELIAL GROWTH

% MANCOZEB	% INHIBITION
0.016	77.7
0.08	87.9
0.5	91.8
1.25	98.5
2.0	100
10.0	100

LETTERS NEXT TO BARS REPRESENT SEPARATION OF MEANS OF RADIAL GROWTH (TUKEY HSD test). STAR SYMBOLS REPRESENT SPORULATION (★ ABSENT; ★★ SPARSE; ★★★ DENSE) and the entomophthtoralean *C. thromboides* were usually highly susceptible to the triazole triadimefon (Majchrowicz & Poprawski, 1993). In assays aimed at investigating the effect on *C. thromboides* and *B. bassiana* of commonly applied fungicides for control of stripe rust, *Puccinia striiformis* f. sp. *tritici* in South Africa, Naudé & Hatting (1998) also found high inhibition by tebuconazole (triazole) and the combination of cyproconazole (triazole) with carbendazim (a carbamate).

Chap 6

The dithiocarbamate mancozeb (Sancozeb) at 0.1% strongly inhibited mycelial growth but not sporulation of *C. thromboides*. Most, if not all, carbamic acid-derived fungicides such as iprodione (a dicarboximide) are slightly harmful or harmful to a wide range of entomopathogenic fungi (Nanne & Radcliffe, 1971; Hassan *et al.*, 1991; Majchrowicz & Poprawski, 1993). Hall & Dunn (1959) showed that zineb at one-third the recommended rate exhibited various effects (from none to complete inhibition) on five entomophthoralean species pathogenic to the spotted alfalfa aphid, *Therioaphis maculata* (Buckton) (Homoptera: Aphididae). Majchrowicz & Mietkiewski (1976) reported the total incompatibility of mancozeb with entomophthoralean and hyphomycetous entomopathogenic fungi. Mancozeb had a fungicidal effect, and zineb and the combination of copper oxychloride + zineb greatly limited growth of entomopathogenic Hyphomycetes and Entomophthorales in laboratory tests (Machowicz-Stefaniak, 1980; Majchrowicz & Poprawski, 1993). Mancozeb also showed considerable inhibition of growth of *B. bassiana* (Clark *et al.*, 1982; Loria *et al.*, 1983), *B. brongniartii* (Vyas *et al.*, 1990), and *V. lecanii* (Hassan *et al.*, 1987), and carbendazim killed *B. brongniartii* (Vyas *et al.*, 1990). Less than 0.5% maneb was harmful to *B. bassiana* and *V. lecanii*, and 0.14% propineb (a dithiocarbamate) killed *B. bassiana* and strongly inhibited *V. lecanii* (Hassan *et al.*, 1991). Maneb (0.5%) and 0.2% propineb also were harmful to *B. brongniartii* in laboratory tests (Coremans-Pelseneer *et al.*, 1991; Coremans-Pelseneer, 1994). Benomyl (a benzimidazolecarbamate) inhibited the development of the Hyphomycetes *B. bassiana*, *Metarhizium anisopliae* (Metschnikoff) Sorokin, *Nomuraea rileyi* (Farlow) Samson, and *V. lecanii* (Wilding, 1972; Ignoffo *et al.*, 1975; Horton *et al.*, 1980; Tedders, 1981; Terribile & Monteiro de Barros, 1992). Many of the carbamate fungicides are in wide use today.

Entomopathogenic fungi are usually susceptible to fungicides (Roberts & Campbell 1977; Galani, 1980; Machowicz-Stefaniak, 1980, 1981; Naudé & Hatting, 1998). Data reported here showed that the susceptibility of *C. thromboides* to fungicides varied. Wilding

& Brobyn (1980) suggested that under field conditions the action of fungicides on the infectivity of aphids by Entomophthorales might influence the germination of conidia. This hypothesis was supported by Delorme & Fritz (1981) for the foliar fungicides sulphur, maneb and mancozeb. Indeed, these fungicides reacting with cell thiols inhibited the use of lipidic supplies and thus hindered spore germination (Fritz, 1977). That there is so little known on the mode of action of fungicides on Entomophthorales and other Zygomycetes is due to the fact that no members of these groups are phytopathogens, a few *Mucor* species excepted. No published supporting data could be found to suggest possible reasons for the variation in response of *C. thromboides* to the different fungicides. Similarly, variations in sensitivity toward a particular fungicide *in vitro* were found, but not explained, even among isolates of the same fungal species (*B. bassiana* and *V. lecanii)* by Olmert & Kenneth (1974).

No published data were found on the effects of the combinations mancozeb + oxadixyl, and captab (a dicarboximide) + metalaxyl on entomopathogenic fungi. The carbamates mancozeb and captab are discussed above, under Sancozeb.

In addition to the abovementioned cases where fungicides (*i.e.*, copper-based compounds, triazoles and carbamates) adversely affect entomopathogenic fungi, some instances have been reported of herbicides (Poprawski & Majchrowicz, 1995) and fungicides enhancing their activity. Loria *et al.* (1983) found that the fungicide metalaxyl (an acylalanine component in Apron C) had a neutral or stimulatory effect *in vitro* on *B. bassiana*. Majchrowicz & Poprawski (1993) found that metalaxyl adversely affected certain Entomophthorales (including *C. thromboides*) and Hyphomycetes, but that it also enhanced the activity of several other fungi. Naudé & Hatting (1998) showed complete compatability of the herbicide metsulfuron with *B. bassiana*.

Clearly, there was a reasonable mancozeb inhibition of hyphal elongation, but it did not seem to be as effective against the elongation of conidiophores as it was against vegetative hyphae. We also may be seeing yet another aspect of the chemical differences between Zygomycetes and the 'higher' fungi (Ascomycetes, Deuteromycetes and Basidiomycetes), where some compounds that are actively inhibitory to the higher fungi and are either ineffective or have modified activities among the Zygomycetes. An example of this is that nystatin has no apparent fungistatic activity against Zygomycetes, thus making it a suitable additive for a selective medium to suppress Hyphomycetes while allowing growth by the Entomophthorales (Dr. Richard Humber, USDA-ARSEF, pers. comm.).

3.5 Summary

Sancozeb at a rate of 1.625% a.i. (20g Sancozeb per litre of water) was used to treat the fungus-contaminated rearing facilities at ARC-SGI. In addition, all greenhouses used for this purpose were washed with water and then fumigated with potassium permanganate ($KMnO_4$) and formaldehyde (4.3 litres formaldehyde plus 1.8 kg $KMnO_4$ for treatment of 283 cubic metres). All cages were washed with the foaming detergent/sanitiser Asepto Z (Klenzade SA (Pty) Ltd, South Africa), a chlorinated alkaline with a concentration of 1-1.5%.

Actively-feeding field aphids were collected to initiate new colonies on a small scale prior to moving the aphids into the larger insectaries inside the treated greenhouses. As soon as Russian wheat aphids were introduced into the cages, the plants, aphids, and soil surface were sprayed with Sancozeb using a hand held-sprayer. At each aphid cycle (*ca.* 10 days), the cages were again washed with Asepto Z, with the new plants, aphids, and soil surface sprayed with Sancozeb.

Despite fumigation, sanitation, and reduction in humidity levels, without Sancozeb, epizootics continued to develop in the control greenhouse. This indicated that the fungicide, not mere fumigation and sanitation, was responsible for control of *C. thromboides* in the validation study.

CHAPTER 4

DISTRIBUTION AND TAXONOMY OF FUNGAL PATHOGENS OF APHIDS FROM SOUTH AFRICA, WITH SPECIAL REFERENCE TO CEREAL APHIDS

4.1 Introduction

The Small Grain Institute (SGI) of the Agricultural Research Council (ARC) of South Africa initiated a wheat-breeding programme after the first host-plant resistance to the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), was reported in South Africa by Du Toit (1987). This new approach toward managing the Russian wheat aphid presented an opportunity for the establishment of an integrated-control programme based on host-plant resistance and natural enemies (Prinsloo *et al.*, 1995, 1997a; Marasas *et al.*, 1997). To date ARC-SGI has released six resistant cultivars (Prinsloo & Tolmay, 1999a, 2002) making use of the major resistance gene DN1 (Marasas, 1999) from the (antibiotic) donor PI 137739 (Du Toit, 1987, 1989a, 1989b). According to projections by Marasas (1999), resistant cultivars released by ARC-SGI would have covered 46.7 and 54.5%, respectively, of areas planted to wheat in the central and eastern Free State province, South Africa, during the year 2000. This scenario implies a dramatic increase in the selection pressure being exerted upon field populations of the Russian wheat aphid and subsequently the possibility for the formation of a resistance-breaking biotype (Prinsloo *et al.*, 1995). It is believed at ARC-SGI that a well-established and diverse natural-enemy complex will safeguard the South African wheat industry by preventing this phenomenon from occurring.

Following the discovery of the Russian wheat aphid near Muleshoe, Texas, in 1986 (Webster & Treat, 1997), it has been intensively researched as a potential candidate for biological control using fungal entomopathogens in the U.S. (Poprawski & Wraight, 1998). However, in South Africa, since first being reported in 1978 (Walters *et al.*, 1980), research on the natural-enemy complex of the Russian wheat aphid has been devoted to the occurrence and impact only of predators and parasitoids (Walters *et al.*, 1980; Du Toit, 1983; Aalbersberg *et al.*, 1984, 1988b; Aalbersberg, 1987; Prinsloo, 1990; Prinsloo & Neser, 1994; Marasas *et al.*, 1997; Marasas, 1999). These research endeavours have subsequently aided in the baseline characterization of the indigenous aphid predator and parasitoid species complexes associated with the Russian wheat aphid in South Africa. Similar survey-type investigations regarding cereal aphid-pathogenic fungi from South Africa have, however, not yet been conducted, with only two reported observations of fungi attacking cereal aphids in the field (Walters *et al.*, 1980; Von Wechmar *et al.*, 1991). Elsewhere entomopathogenic fungi often cause natural epizootics among aphid populations, including those of the Russian wheat aphid (Feng *et al.*, 1990a, 1991a, 1992b; Wraight *et al.*, 1993; Wanjama & Arama, 1999). Several insect fungi are considered by some as promising natural enemies for aphid biological control (Latgé & Papierok, 1988; Humber, 1991; Milner, 1997).

Due to the very limited information available regarding the indigenous insect-pathogenic fungi of South Africa, a series of surveys was conducted during 1995 through 1999 in a first attempt to characterize the South African species complex of fungal pathogens of cereal aphids. These surveys also presented the opportunity to collect fungal species and strains occurring under extreme environmental conditions. This is important in screening for fungal strains with below-normal temperature optima and therefore with an ability to infect early-season populations of the Russian wheat aphid (Goettel *et al.*, 1993; Wraight *et al.*, 1993). Surveys were extended to include nonagricultural aphid hosts to gain a better understanding of the seasonal distribution, host range, and survival strategies of the various fungi. Findings would ultimately provide a baseline for a strategy on the use of fungal pathogens in the integrated-control programme against this very important pest.

4.2 Material and methods

4.2.1 Collection sites and protocol

Surveys were conducted in the Free State, KwaZulu-Natal, Western Cape and northern Eastern Cape, and Gauteng Provinces of South Africa. A total of 13 sites were surveyed throughout these regions (Fig. 4.1). Emphasis was placed on surveying the small-grain aphid complex occurring in both the summer rainfall (dryland and irrigation) and winter rainfall (dryland) wheat-production areas.

Dryland wheat (*Triticum aestivum* L.), oats (*Avena sativa* L.), and triticale (X *Triticosecale rimpaui* Wittm.) were surveyed at ARC-SGI, Bethlehem, Free State. The only irrigated wheat surveyed in the summer-rainfall region was in the Winterton/Bergville area of KwaZulu-Natal where all wheat is produced under central-pivot irrigation systems. Small grains surveyed in the Western Cape (winter-rainfall) included dryland wheat and oats.

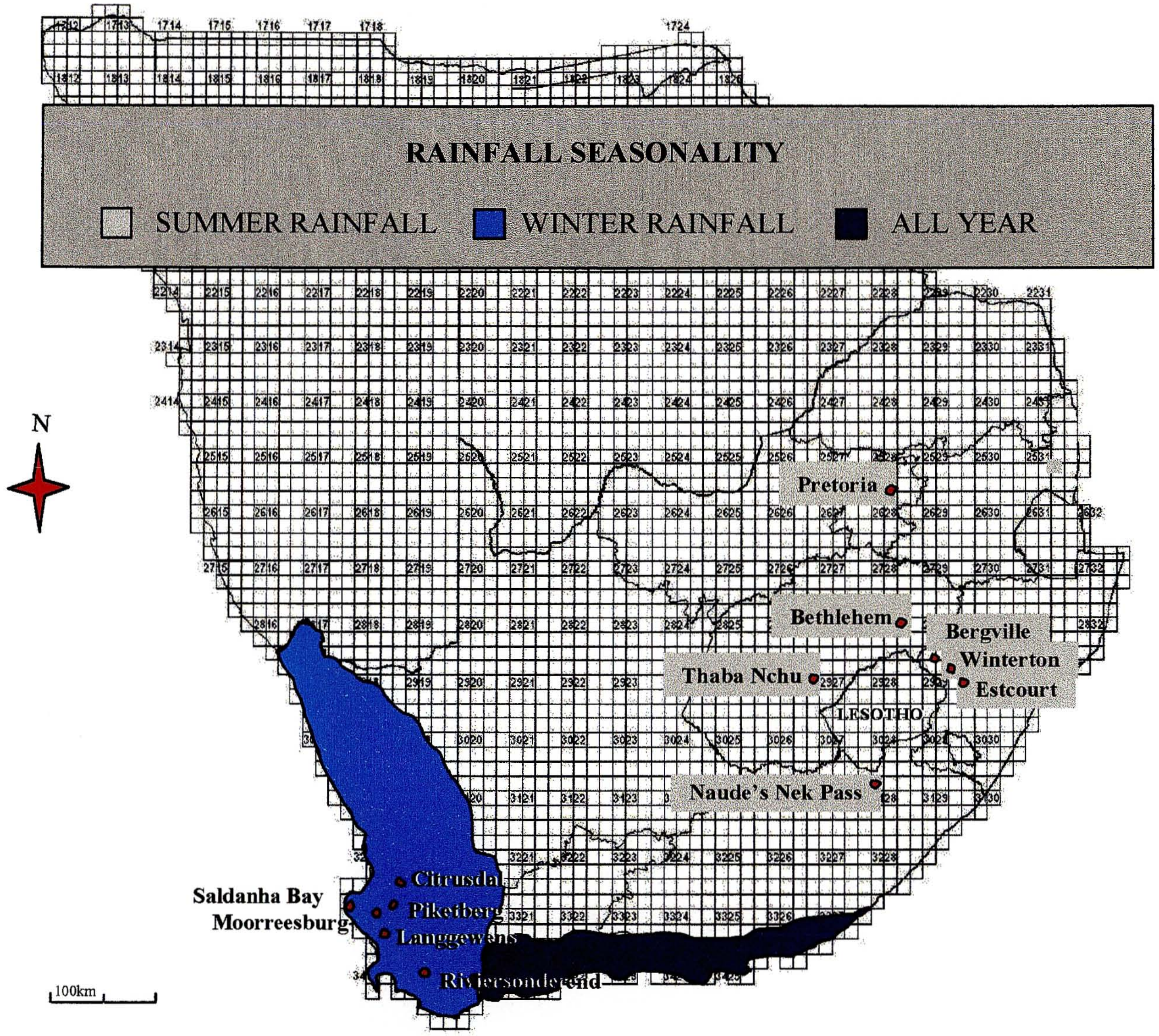


Figure 4.1. Sampling sites for aphids and associated entomopathogenic fungi in South Africa, 1995 to 1999. (Rainfall demarcation according to Schulze, 1997)

Several nonagricultural host plants were surveyed in the Free State and KwaZulu-Natal and isolated collections were made in the Western Cape, northern Eastern Cape, and Gauteng.

In the Bergville/Winterton area, KwaZulu-Natal, surveys were performed at 10 equally spaced points 2 m apart along five parallel transects (5 m apart), totalling 50 tillers (collected) per field surveyed. At Langgewens, Western Cape, and Bethlehem, Free State, individual flag leaves (sample units) showing typical Russian wheat aphid damage (see Chapter 1, section 1.2) were randomly collected. All sample units collected were at least 1 m apart. Cadavers of nonagricultural aphid hosts were collected on a purely random basis, sometimes fortuitously; if plants were found infested with aphids, the aphid colony was simply searched and cadavers collected.

Collections of aphid cadavers were made to (1) identify the aphid host and etiologic agent of disease, and (2) preserve live fungal material for later isolation and culturing in or on artificial media. Dead aphids, with or without external signs of mycosis (*i.e.*, abnormalities in the morphology, colour variation, conspicuous position of appendages such as rhizoids and cystidia), were collected with a piece of substrate to which they adhered. This procedure would aid in the identification of the fungus through the presence/absence and morphological characteristics of specialized structures, *e.g.*, rhizoids attaching the cadaver to the substrate or cystidia that help the conidiophores to emerge from the host body. The substrate with attached cadaver was placed in either a plastic zip-lock bag, empty plastic 35 mm-film container or, if only a few cadavers were available for collection, in 70% ethanol inside a 2ml Eppendorf® safe-lock tube (Merck NT Laborotory Supplies (Pty) Ltd. Bloemfontein, South Africa. Cat no. 306/0314/02) for safe preservation. The collected material was transported promptly to the laboratory for further study. When transported over a long distance, the collections were kept cool by placing them inside a cooler box with one or two freezer blocks.

4.2.2 Isolation of fungi

Aphid cadavers not yet showing any sign of external vegetative growth or sporulation were surface-sterilized in a 1% sodium hypochlorite solution for 30 seconds and then passed through a series of three washes in sterile distilled water. Immediately after washing, cadavers were placed on 2% water agar in sealed Petri dishes and incubated at 20-22°C until external fungal growth and/or sporulation was visible. When ballistospores, rhizoids, and/or cystidia were observed on a cadaver, either: (1) surface-sterilized cadavers were suspended directly above the culture medium by attaching the cadaver to a small ball of petroleum jelly placed on the underside of the Petri dish lid, thereby allowing discharged conidia to 'shower' onto the medium below; (2) the Petri dish with culture medium and attached cadaver was placed upside down allowing discharged conidia to shoot upwards onto the culture medium; or (3) the cadaver was suspended above the medium by inserting the sharp end of a sterile 'minuten' insect pin through the thorax of the aphid cadaver and inserting the other end in the culture medium at an angle of 45° using forceps. The Petri dishes were sealed with Parafilm and incubated in a 4301 growth chamber at 20-22°C with a photoperiod of 16:8 (L:D) h until sporulation was observed. After sufficient sporulation, the Petri dish lid was replaced or the pin with attached cadaver was removed. When attempting to isolate the fungus from a cadaver that was already actively sporulating, the upwards showering method was generally used. For cadavers that were covered with white mycelial growth and did not produce rhizoids, cystidia, or ballistospores, a small quantity of inoculum was scraped from the cadaver with a sterile needle and transferred to an appropriate agar medium.

All hyphomycetous and some entomophthoralean isolates were grown on Sabouraud dextrose agar (Biolab Diagnostics, Midrand, Halfway House, South Africa) supplemented with 1% yeast extract (SDAY) (Difco Laboratories, Detroit, Michigan) at pH 6.7. Other media yielding good fungal growth were potato dextrose agar (PDA) (Biolab Diagnostics), a wheat-germ medium (Srinivasan *et al.*, 1964), and phytone yeast-extract agar (PYE) (Carmichael, 1962).

Two fastidious entomophthoralean species were isolated on Sabouraud egg-yolk and milk agar (SEMA) (4 g peptone, 16 g maltose, 6 g agar, 56 ml fresh cows' milk, 43 ml egg yolk, 2 ml wheat germ oil, and 400 ml distilled water; pH 6.7). One entomophthoralean species was isolated by transferring a surface-sterilized cadaver into 10 ml of Grace's insect tissue culture medium (Sigma Chemical Co., St. Louis, Missouri) amended with 5% fetal bovine serum (Sigma Chemical Co.) in a Wasserman tube (Merck NT Laborotory Supplies (Pty) Ltd., Gauteng, South Africa). The aphid body was ruptured with a sterile needle to release fungal inoculum into the liquid.

No antibiotics were added to any of the media. As far as possible, isolates were cultured at 20-22°C with a photoperiod of 16:8 (L:D) h under saturated humidity conditions.

4.2.3 Identification of fungi

Fresh and desiccated cadavers were placed on 1.5% water agar in a Petri dish for 6-12 h to induce sporulation. Sporulating cadavers were examined under a dissecting microscope for evidence of rhizoids, cystidia, mycelia, and/or spores. Special attention was given to the mode of attachment, if any, of the cadaver to the substrate, *i.e.*, proboscis versus rhizoids. For semipermanent mounts, fungal structures were stained with 2% aceto-carmine (2 g carmine extracted in 100 ml of 45% acetic acid) and mounted in a drop of lactophenol (Humber, 1997). For permanent mounts, fungal structures stained with 2% aceto-carmine, were mounted in a drop of Canada balsam according to the method of Pienaar (1955), using Meyer's albumin for preparation of the cover slides (Darlington & La Cour, 1976).

The fungi were identified according to the keys in Zimmermann (1978), Waterhouse & Brady (1982), Balazy (1993), and Humber (1997). All conidial structures measured were produced *in vivo* unless otherwise stated. Conidial measurements are expressed as (minimum length) - mean length - (maximum length) x (minimum width) - mean width - (maximum width), unless otherwise stated. Vouchers of pure cultures established *in vitro* and of dried specimens were deposited in the South African National Collection of Fungi (SANCF), ARC-Plant Protection Research Institute (PPRI), Pretoria, and in the United States Department of Agriculture Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, New York. The codens used by SANCF are 'PPRI' for live cultures and 'PREM' for dried specimens. The 'ARSEF' coden is used for both live cultures (four digit accession number) and dried specimens (nine digit accession number). All codens are registered with the World Federation of Culture Collections' World Data Center for Microbiology (see http://wdcm.nig.ac.jp/) and with the International Association of Plant Taxonomists' Index Herbariorum (see http://www.nybg.org/bsci/ih/ih.html).

4.3 Results

Sixteen aphid species were collected from which six Entomophthorales and three Hyphomycetes, known to infect and kill insect hosts, were recorded. The cereal-aphid complex from which mycosed aphids were recorded comprised these species: Russian wheat aphid, *D. noxia*; bird-cherry oat aphid, *Rhopalosiphum padi* (L.); corn leaf aphid, *Rhopalosiphum maidis* (Fitch); rose-grain aphid, *Metopolophium dirhodum* (Walker); and brown wheat-ear aphid, *Sitobion avenae* (F.) (Table 4.1). No greenbug, *Schizaphis graminum*

Table 4.1. Survey data on insect pathogenic fungi attacking aphid hosts in South Africa, 1995 to 1999

Fungal species	**Aphid host**	**Aphid host plant**	**Locality**	**Grid reference**[a]	**Collection date**
Pandora neoaphidis	*Metopolophium dirhodum*	*Triticum aestivum*	Winterton	S28°48.168'/E29°31.390'	Oct 96
			Bergville	S28°45.632'/E29°18.091'	Oct 96, Sept-Oct 97
			Riviersonderend	S34°09.230'/E19°54.581'	Sept 96, Sept 97
			Langgewens	S33°16.329'/E18°42.484'	Sept 96
			Piketberg	S32°50.865'/E18°46.914'	Sept 97
			Bethlehem	S28°09.756'/E28°17.787'	Nov 96
		Avena sativa	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
		X Triticosecale rimpaui	Bethlehem	S28°09.353'/E28°18.213'	Nov 97
		Bromus catharticus	Bethlehem	S28°09.855'/E28°18.421'	Sept 97
			Naude's Nek Pass	S30°43.865'/E28°06.313'	Mar 97
	Diuraphis noxia	*T. aestivum*	Riviersonderend	S34°09.230'/E19°54.581'	Sept 96, Sept 97
			Bethlehem	S28°09.730'/E28°17.848'	Nov 96
			Bethlehem	S28°09.756'/E28°17.787'	Oct- Nov 97
			Langgewens	S33°16.329'/E18°42.484 '	Sept 97
	Sitobion avenae	*T. aestivum*	Riviersonderend	S34°09.230'/E19°54.581'	Sept 96

Table 4.1. Survey data on insect pathogenic fungi attacking aphid hosts in South Africa, 1995 to 1999 (continued)

Fungal species	**Aphid host**	**Aphid host plant**	**Locality**	**Grid reference**[a]	**Collection date**
Pandora neoaphidis	*Myzus sp.*	*Urtica urens*	Naude's Nek Pass	S30°43.865'/E28°06.313'	Mar 97
	Macrosiphum euphorbiae	*Urtica urens*	Naude's Nek Pass	S30°43.865'/E28°06.313'	Mar 97
	Aphis gossypii	*Cuphea melvilla* [b]	Estcourt	S29°01.086'/E29°52.046'	May 97, Apr 98
	Rhopalosiphum padi	*T. aestivum*	Langgewens	S33°16.329'/E18°42.484'	Sept 97
			Bethlehem	S28°09.736'/E28°17.787'	Nov 97
			Bergville	S28°45.632'/E29°18.091'	Sep 97
		A. sativa	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
	Rhopalosiphum maidis	*T. aestivum*	Bethlehem	S28°09.756'/E28°17.787'	Oct 97
			Riviersonderend	S34°09.230'/E19°54.581'	Sept 96
			Langgewens	S33°16.329'/E18°42.484'	Sept 96
			Winterton	S28°48.168'/E29°31.390'	Oct 96
	Ureleocin sonchi	*Sonchus oleraceus*	Saldanha Bay	S32°59.156'/E17°58.651'	Sept 96
			Citrusdal	S32°37.434'/E18°57.176'	Sept 97
	Melanaphis sacchari	*Sorgum bicolor*	Thaba Nchu	S29°03.865'/E26°55.243'	Mar 98

Table 4.1. Survey data on insect pathogenic fungi attacking aphid hosts in South Africa, 1995 to 1999 (continued)

Fungal species	**Aphid host**	**Aphid host plant**	**Locality**	**Grid reference**[a]	**Collection date**
Conidiobolus thromboides	*D. noxia*	*T. aestivum*	Bethlehem [c]	S28°09.865'/E28°18.579'	Oct 95
	M. dirhodum	*A. sativa*	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
	R. padi	*T. aestivum*	Langgewens	S33°16.329'/E18°42.484'	Sept 97
		A. sativa	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
	S. avenae	*A. sativa*	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
	R. maidis	*A. sativa*	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
Conidiobolus coronatus	*M. dirhodum*	*T. aestivum*	Bethlehem [c]	S28°09.898'/E28°18.535'	Oct 96
Conidiobolus obscurus	*R. padi*	*T. aestivum*	Langgewens	S33°16.329'/E18°42.484'	Sept 97
			Bergville	S28°45.632'/E29°18.091'	Sept 97
	D. noxia	*T. aestivum*	Bethlehem	S28°09.756'/E28°17.787'	Oct 97
		T. aestivum	Bethlehem [c]	S28°09.858'/E28°18.572'	Apr 98
		T aestivum	Riviersonderend	S34°09.230'/E19°54.581'	Sept 97
	M. dirhodum	*T. aestivum*	Bergville	S28°45.632'/E29°18.091'	Oct 96, Sept-Oct 97
		A. sativa	Bethlehem	S28°09.831'/E28°17.385'	Nov 96
	R. maidis	*A. sativa*	Bethlehem	S28°09.831'/E28°17.385'	Nov 96

Table 4.1. Survey data on insect pathogenic fungi attacking aphid hosts in South Africa, 1995 to 1999 (continued)

Fungal species	**Aphid host**	**Aphid host plant**	**Locality**	**Grid reference**[a]	**Collection date**
Conidiobolus obscurus	*S. avenae*	*T. aestivum*	Winterton	S28°48.168'/E29°31.390'	Oct 96
			Bergville	S28°45.632'/E29°18.091'	Sept 97
			Riviersonderend	S34°09.230'/E19°54.581'	Sept 97
Neozygites fresenii	*Chaitophorus populialbae*	*Populus canescens*	Bethlehem	S28°09.967'/E28°18.784'	July 97
	Hyalopterus pruni	*Phragmites australis*	Bethlehem	S28°10.138'/E28°18.530'	Feb 97, Mar 98
	Myzus persicae	*Tropaeolum majus*	Estcourt	S29°01.085'/E29°52.037'	May 97, Apr 98
	A. gossypii	*Cuphea melvilla* [b]	Estcourt	S29°01.086'/E29°52.046'	Apr 98
Entomophthora planchoniana	*H. pruni*	*P. australis*	Bethlehem	S28°10.138'/E28°18.530'	Jan 97, Jan 98
	M. euphorbiae	*Cussonia paniculata* [b]	Bethlehem	S28°13.735'/E28°20.013'	May 97
		Bignonia sp. [b]	Bethlehem	S28°13.518'/E28°19.835'	June 98
		Borago officinalis [b]	Pretoria	S25°48.173'/E28°17.406'	Sept 97
	Myzus ornatus	*Bidens pilosa*	Estcourt	S29°01.080'/E29°52.032'	May 97
	M. persicae	*T. majus*	Estcourt	S29°01.085'/E29°52.037'	May 97, Apr 98, June 98
	Aphis spiraecola	*U. urens* [b]	Bethlehem	S28°14.926'/E28°19.133'	Sept 97
		Cotoneaster franchetii	Bethlehem	S28°13.518'/E28°19.835'	June 98

Table 4.1. Survey data on insect pathogenic fungi attacking aphid hosts in South Africa, 1995 to 1999 (continued)

Fungal species	**Aphid host**	**Aphid host plant**	**Locality**	**Grid reference**[a]	**Collection date**
Entomophthora planchoniana	*Sitobion fragariae*	*Lactuca serriola*[b]	Bethlehem	S28°09.865'/E28°18.375'	Oct 97
	D. noxia	*T. aestivum*	Bethlehem	S28°09.756'/E28°17.787'	Nov 97
	A. gossypii	*Ipomoea purpurea*[b]	Estcourt	S29°01.086'/E29°52.040'	Apr 98
Verticillium lecanii	*D. noxia*	*T. aestivum*	Moorreesburg	S33°06.432'/E18°40.967'	Sept 96
Beauveria bassiana	*D. noxia*	*T. aestivum*	Langgewens	S33°16.329'/E18°42.484'	Sept 97
Paecilomyces farinosus	*D. noxia*	*T. aestivum*	Bethlehem[c]	S28°09.865'/E28°18.579'	June 99

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[a] Grid references were recorded with a Garmin® Global Positioning System II, using the 1984 World Geodetic System (WGS 84) as map datum.

[b] New aphid-host plant association in South Africa.

[c] In greenhouse at the Small Grain Institute.

(Rondani), cadavers were found during the surveys. What follows is a description of the insect-pathogenic fungal species recorded from various aphid hosts during this study.

Pandora neoaphidis (Remaudière & Hennebert) Humber (Zygomycetes). Field collections: recorded from ten aphid host species in 11 locations. Freshly killed aphids typically pale brown or brick red in colour turning a rusty red colour upon desiccation. Cadavers attached to the substrate by a few single rhizoids generally emerging from the aphid's abdomen and/or thorax. Isolation: two isolates established on SEMA and Grace's insect tissue-culture medium amended with 5% fetal bovine serum. Both isolates from *M. dirhodum* cadavers collected at Riviersonderend, Western Cape (4 Sept. 1997). Description: primary conidia (17.0)-20.92-(27.0) x (9.5)-10.97-(13.0) µm (n = 50); generally clavate to obovoid; uninucleate with basal papilla displaced laterally from the spore axis (Fig. 4.2a); forcibly discharged by papillary eversion, often creating a white halo around the cadaver. Secondary conidia: produced singly on primary conidia; similar to or more nearly globose than the latter (Fig. 4.2b). Conidiophores: digitately branched at their apices (Fig. 4.2c). Cystidia: distally tapering, 2-3 x thicker than conidiophores (Fig. 4.2d); generally produced before the formation of the hymenium. Rhizoids: 2-3 x diameter of hyphae; each ending in a discoid-like expansion (Fig. 4.2e). Resting spores not observed. Diagnosis: primary conidia clavate to obovoid; basal papilla displaced laterally from spore axis; digitately branched conidiophores; distally tapering cystidia, 2-3 x thicker than conidiophores; rhizoids 2-3 x thicker than conidiophores; cadaver attached to substrate by discoid holdfast. Accession nos.: PPRI 6691; PREM 55365, 55862, 55863, 55864 & 55865; ARSEF 5707, 5708, 199804001, 199804002 & 199804003.

Conidiobolus thromboides Drechsler (Zygomycetes). Initially isolated from *D. noxia*, following large scale epizootics occurring in greenhouse rearings at the SGI. Field collections: identified from five cereal-aphid species (Table 4.1); cadavers attached to the substrate by their proboscises (Fig. 4.3a). Fresh cadavers characterized by rusty brown colour developing around the point of entry of the conidial germ tube with tissue necrosis spreading quickly to the rest of the body resulting in the cadaver becoming a red-brown colour within 12 h post mortem; sporulating cadavers white to creamy-white in colour. Isolation: the isolate causing epizootics within *D. noxia* rearings at the SGI was successfully isolated on a wheat-germ medium and SDAY. Description: primary conidia (17.0)-19.56-(23.0) x (13.0)-15.77-(19.5) µm (n = 50); distinctly pyriform with a basal papilla emerging gradually from the

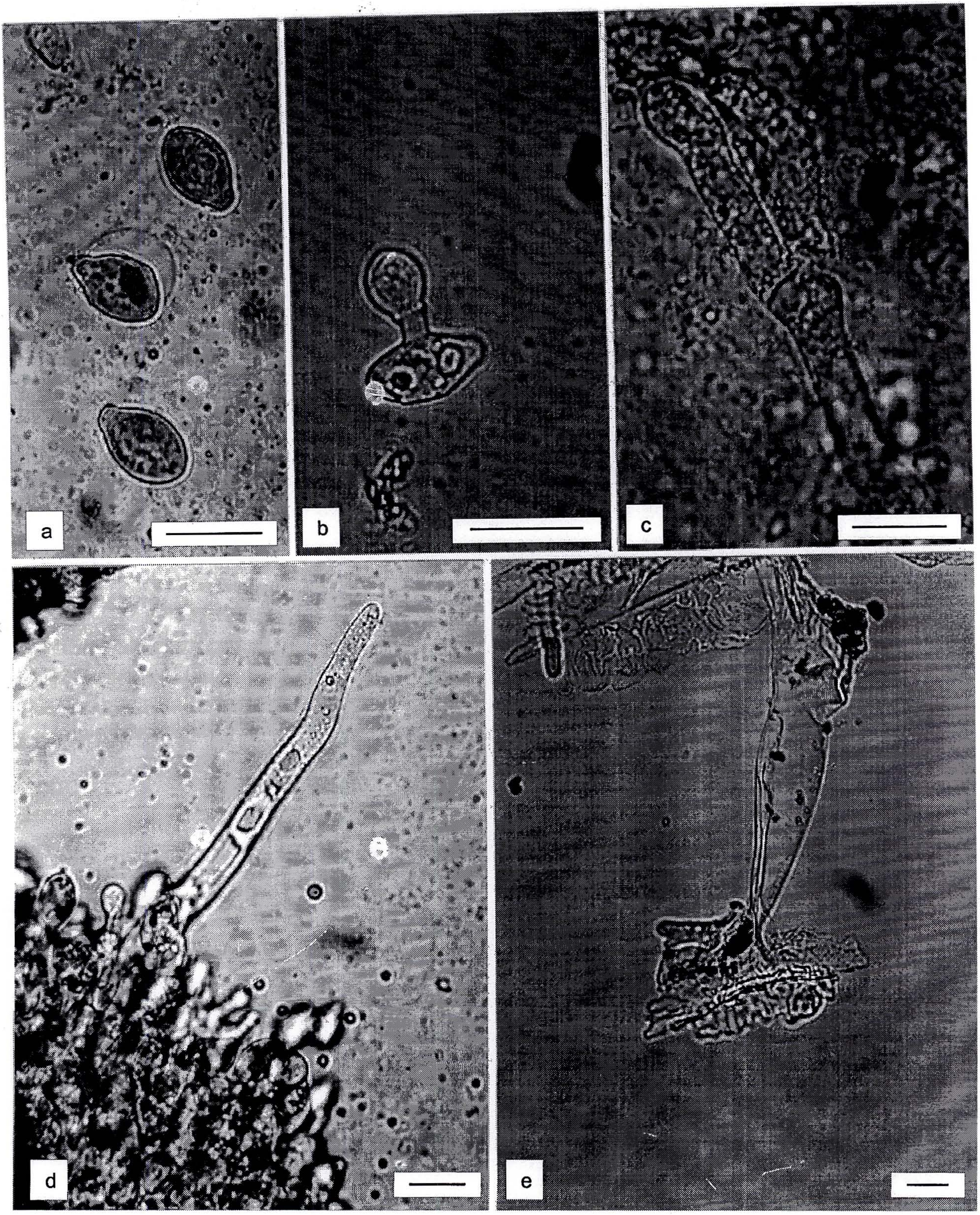

Figure 4.2. *Pandora neoaphidis.* (a) Primary conidia with basal papilla displaced laterally from spore axis. (b) Nearly globose secondary conidium (top) produced on primary conidium. (c) Digitately branched conidiophore. (d) Distally tapering cystidium projecting from hymenium. (e) Rhizoid ending in discoid-like holdfast. Bars = 20 μm.

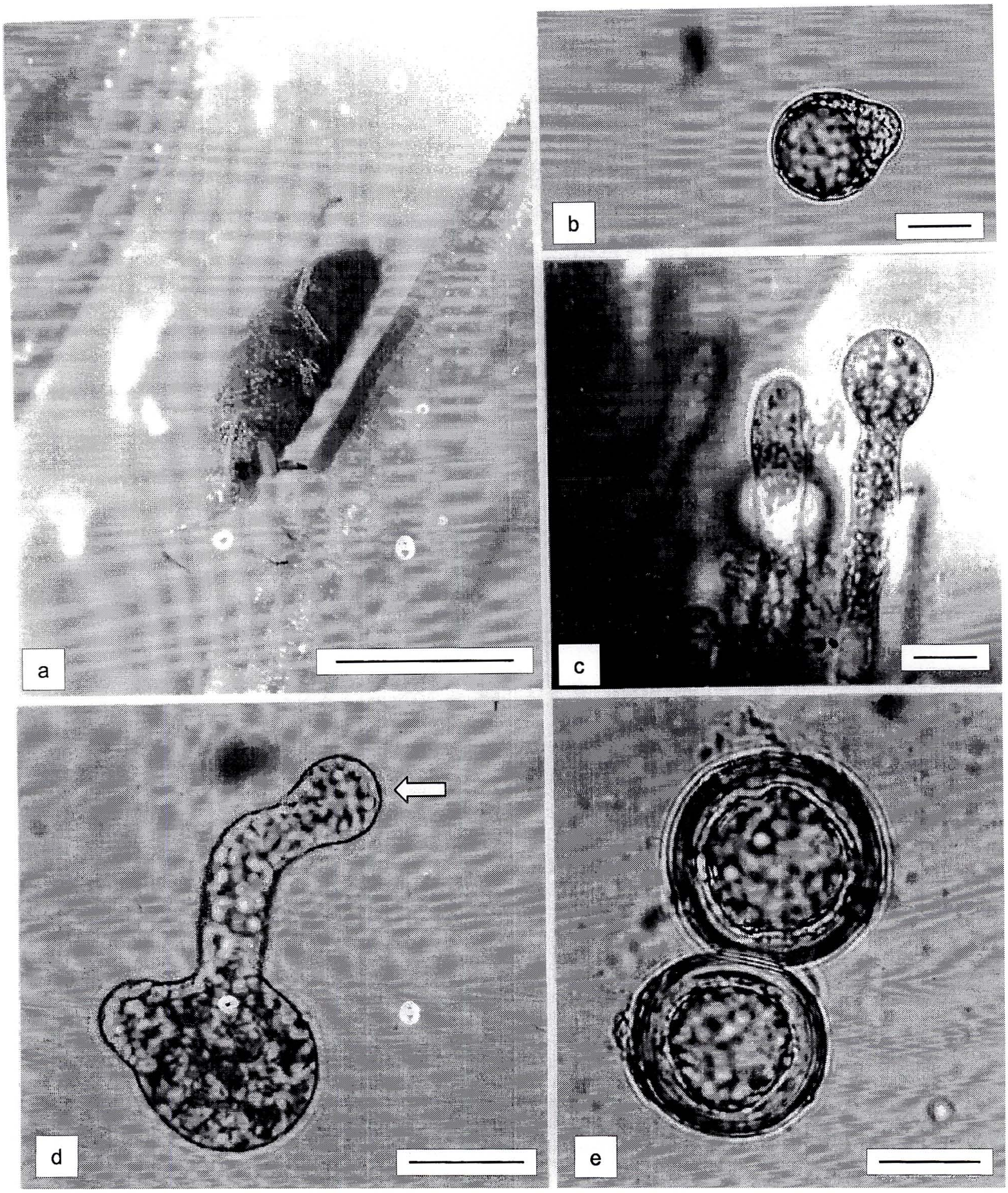

Figure 4.3. *Conidiobolus thromboides*. (a) Freshly-killed *Diuraphis noxia* cadaver attached to substrate by proboscis (Bar = 1 mm). (b) Pyriform primary conidium with basal papilla emerging gradually from spore outline. (c) Primary conidium carried on simple unbranched conidiophore. (d) Secondary conidium (arrow) developing on germ tube arising from primary conidium. (e) Double-walled resting spores. Bars = 10 μm.

spore outline (Fig. 4.3b); forcibly discharged from simple unbranched conidiophores (Fig. 4.3c). Secondary conidia: produced on a germ tube arising from the primary conidia (Fig. 4.3d). Resting spores: typically double walled (Fig. 4.3e), with average diameter 21.07 µm (n = 50) (*in vitro*); resting spores observed from a single *R. padi* cadaver collected on *A. sativa* at SGI (21 Nov. 1996); cadaver rusty-brown colour. Diagnosis: small pyriform conidia (17-23 x 13-20 µm in diameter) with basal papilla emerging gradually from the spore outline; no capilliconidia or microconidia formed; conidiophores unbranched. Accession nos.: PPRI 6014; PREM 55366; ARSEF 199804010.

Conidiobolus coronatus (Costantin) Batko (Zygomycetes). Field collections: no record from field collected cadavers; collected from a greenhouse colony of *M. dirhodum* at the SGI (Table 4.1); single mycosed *M. dirhodum* cadavers were found on the soil surface of potted wheat plants within the aphid-rearing regimen. Isolation: pure cultures established on SDAY and PDA. Description: primary conidia globose, closely resembling *Conidiobolus obscurus* although slightly larger and with more prominent basal papilla tapering toward pointed apex; varying in range (41.5)-47.5-(66.0) x (30.0)-38.97-(48.0) µm (n = 50) (*in vitro*); basal papilla prominent with pointed apex (Fig. 4.4a), averaging 14.18 µm long (n = 40) (*in vitro*); forcibly discharged. Secondary conidia: forming singly and forcibly discharged or, commonly, producing many forcibly discharged secondary microconidia on short germ tubes arising from primary conidia (Fig. 4.4b). Conidiophores: simple unbranched. Resting spores: resembling primary conidia but covered with villose appendages (Fig. 4.4c). Diagnosis: villose resting spores unique to this species; primary conidia large, 40-65 x 30-50 µm in diameter; basal papilla relatively long, emerging abruptly from the spore outline with pointed apex; secondary microconidia readily produced *in vitro*. Accession no.: PREM 55919.

Conidiobolus obscurus (Hall & Dunn) Remaudière & Keller (Zygomycetes). Field collections: recorded throughout all the small-grain production areas surveyed and identified from five cereal-aphid species (Table 4.1). Mode of attachment of cadaver to substrate (*i.e.*, proboscis) and colour of sporulating cadaver resembling those killed by *C. thromboides*. Isolation: pure cultures isolated from *D. noxia* established on PYE and SEMA. Description: primary conidia (30.0)-35.79-(42.0) x (27.0)-30.99-(36.0) µm (n = 50); basal hemispherical papilla, emerging abruptly from the spore outline (Fig. 4.5a); forcibly discharged. Secondary conidia: morphologically similar to primary conidia; produced on a short germ tube arising from primary conidia (Fig. 4.5b). Conidiophores: simple, unbranched (Fig. 4.5c). Resting

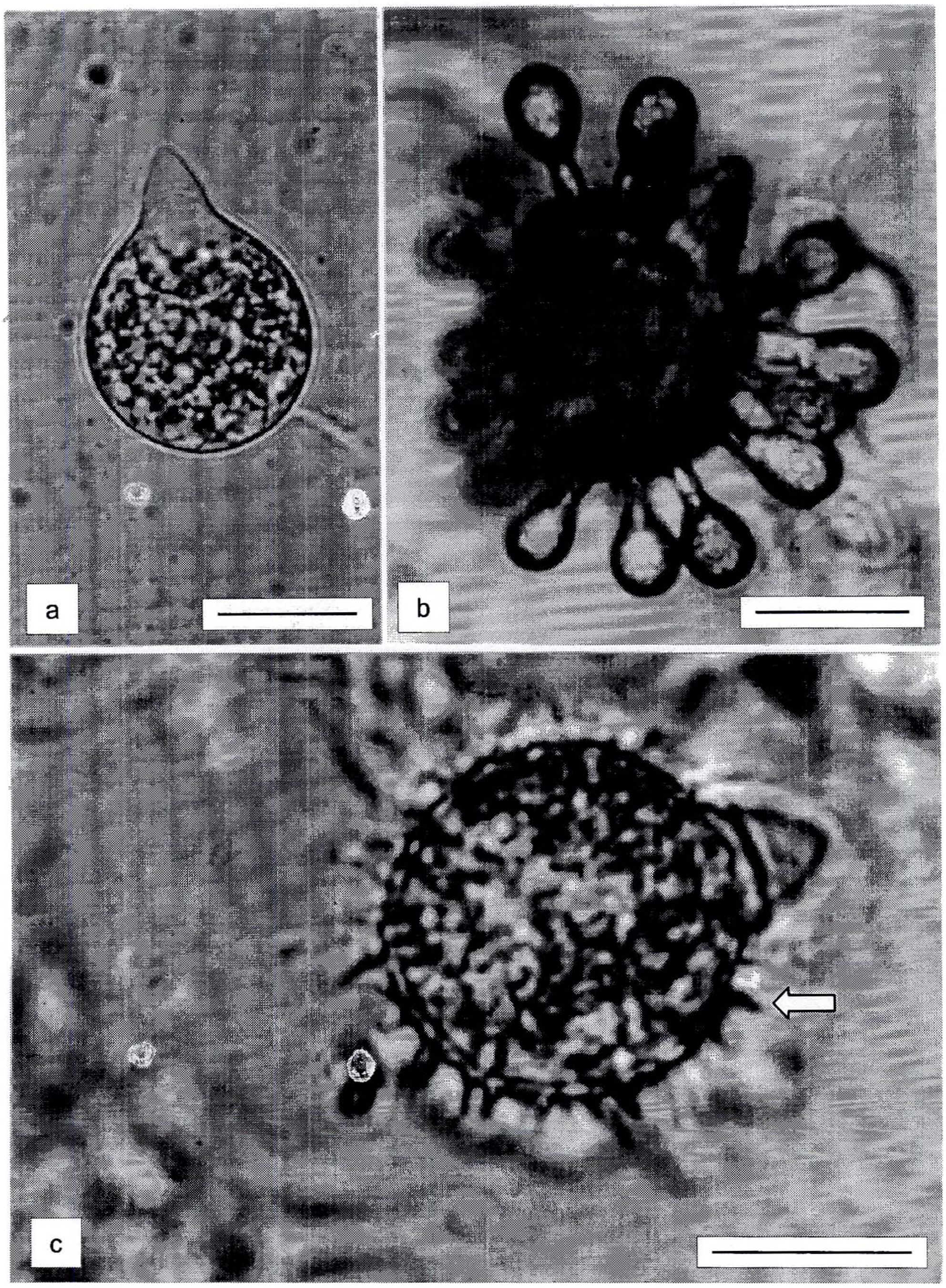

Figure 4.4. *Conidiobolus coronatus*. (a) Primary conidium with relatively long pointed papilla. (b) Microconidia produced on short germ tubes arising from primary conidium. (c) Resting spore covered with villose appendages (arrow). Bars = 20 µm.

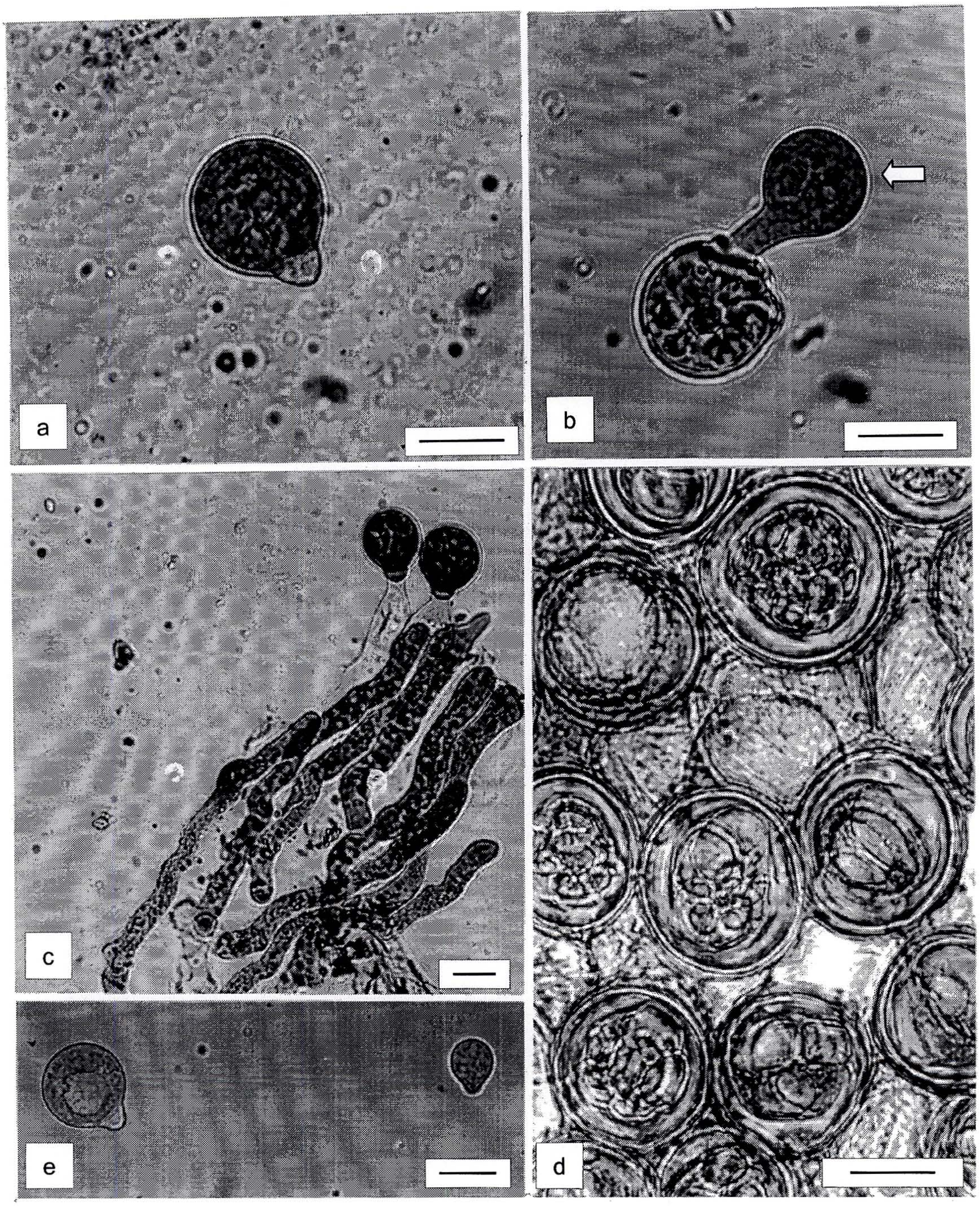

Figure 4.5. *Conidiobolus obscurus.* (a) Primary conidium with basal hemispherical papilla emerging abruptly from spore outline. (b) Secondary conidium (arrow) produced on germ tube arising from primary conidium. (c) Simple unbranched conidiophores. (d) Double-walled resting spores. (e) Comparative size of *C. obscurus* (left) and *C. thromboides*. Bars = 20 µm.

spores: similar to those produced by *C. thromboides* although much larger in size, averaging 34.82 µm (n = 50) in diameter (Fig. 4.5d); resting spores identified from a single *R. padi* cadaver collected in the Western Cape during September 1997. Diagnosis: primary conidia substantially larger (30-35 µm in diameter) than those produced by *C. thromboides* (Fig. 4.5e); basal papilla hemispherical, emerging abruptly from the spore outline. Accession nos.: PPRI 6714; PREM 55868; ARSEF 199804007, 199804009 & 199804014.

Neozygites fresenii (Nowakowski) Remaudière & Keller (Zygomycetes). Field collections: identified from four aphid species (Table 4.1). Mycosed aphids typically dark brown to grey with a shade of violet in colour; attached to substrate by proboscis. Isolation: not cultured *in vitro*. Description: primary conidia (15.5)-17.71-(20.5) x (12.5)-14.83-(16.5) µm (n = 50); nearly spherical to ovoid with a flattened basal papilla (Fig. 4.6a); forcibly discharged. Secondary conidia: capilliconidia (19.0)-23.54-(29.0) x (10.0)-13.4-(16.0) µm (n = 50); carried on capillary conidiophores arising from primary conidia; passively discharged from capillary conidiophores; almond-shaped with a mucoid drop at the tip (Fig. 4.6b). Conidiophores: simple unbranched; many cadavers found with capillary conidiophores extending from the body. Resting spores: black to smoky-grey in colour; ovoid; arising from conjugation between two spherical gametangia (Fig. 4.6c); (23.5)-27.3-(30.0) x (17.5)-20.77-(23.0) µm (n = 30); observed from several *Chaitophorus populialbae* (Boyer de Foscolombe) cadavers collected at SGI (1 July 1996); cadavers typically black in colour and concentrated in groups of up to 20 on the lower surface of *Populus canescens* (Ait.) J. E. Sm. leaves at the branching of the petiole (Fig. 4.6d). Diagnosis: primary conidia subglobose, 15-21 x 12-17 µm; forcibly discharged. Secondary conidia: almond-shaped capilliconidia with mucoid apical droplet; carried on capillary conidiophores arising from primary conidia; passively discharged. Resting spores: dark coloured zygospores; 23-30 x 17-23 µm. Accession nos.: PREM 55866 & 55869; ARSEF 199804005 & 199804011.

Entomophthora planchoniana Cornu (Zygomycetes). Field collections: identified from eight aphid species including *D. noxia* (Table 4.1). Aphid cadavers attached to the substrate by many rhizoids. Freshly killed aphids typically brick red in colour becoming a pale brown colour upon sporulation. Isolation: limited growth obtained on SEMA following a direct spore shower of the medium from a surface-sterilized *Hyalopterus pruni* (Geoffroy) cadaver; the culture could not be maintained despite attempts at transferring inoculum to Grace's and other media. Description: primary conidia (16.0)-18.48-(22.0) x (13.9)-15.54-(19.0) µm (n =

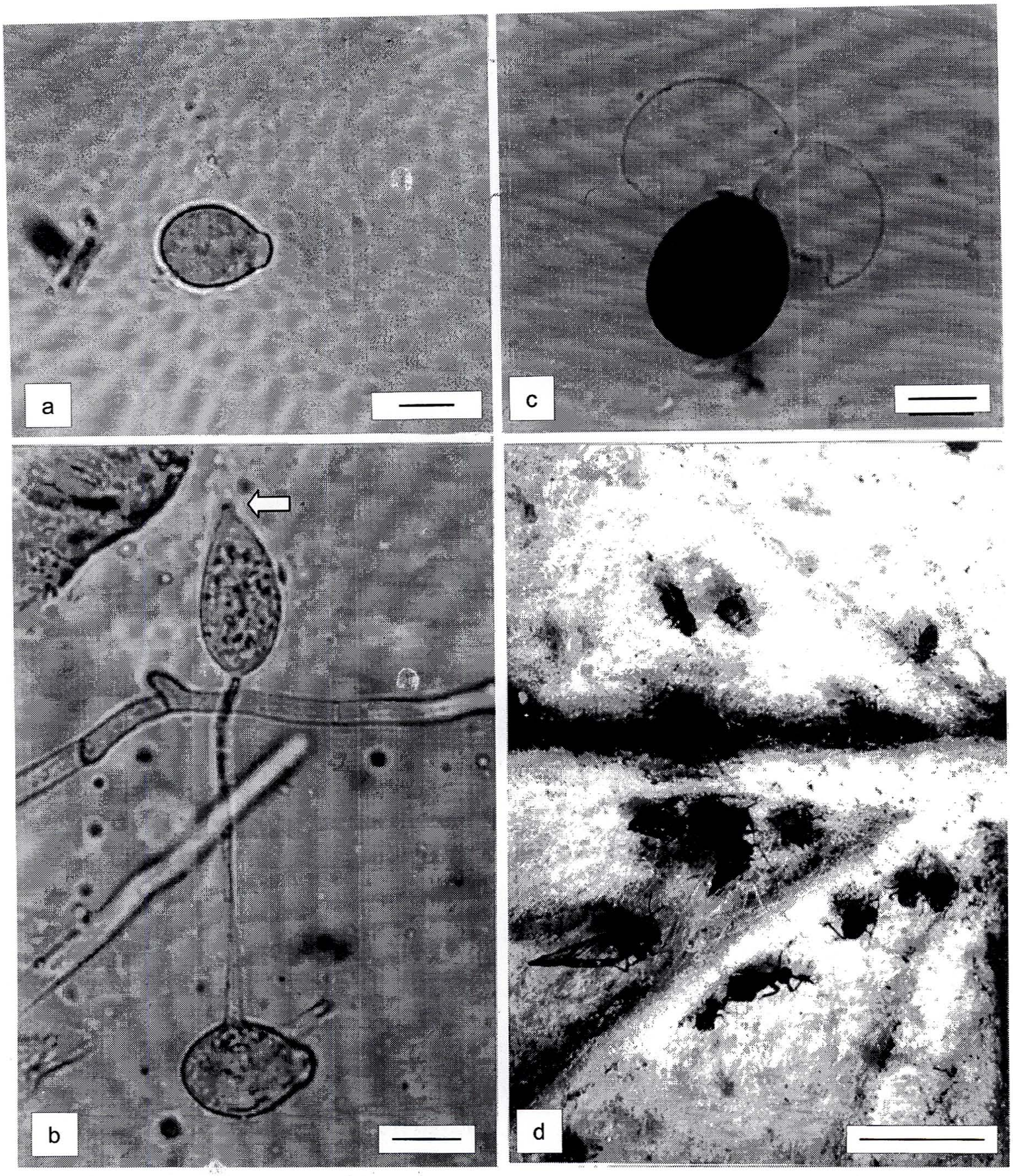

Figure 4.6. *Neozygites fresenii.* (a) Primary conidium with flattened basal papilla. (b) Secondary capilliconidium with mucoid droplet (arrow) produced on capillary conidiophore arising from primary conidium. (c) Resting spore with transparent remnants (top) of gametangia. (d) *Chaitophorus populialbae* cadavers filled with resting spores, concentrated on the lower surface of a *Populus canescens* leaf at the branching of the petiole (Bar = 2 mm). Bars = 10 µm.

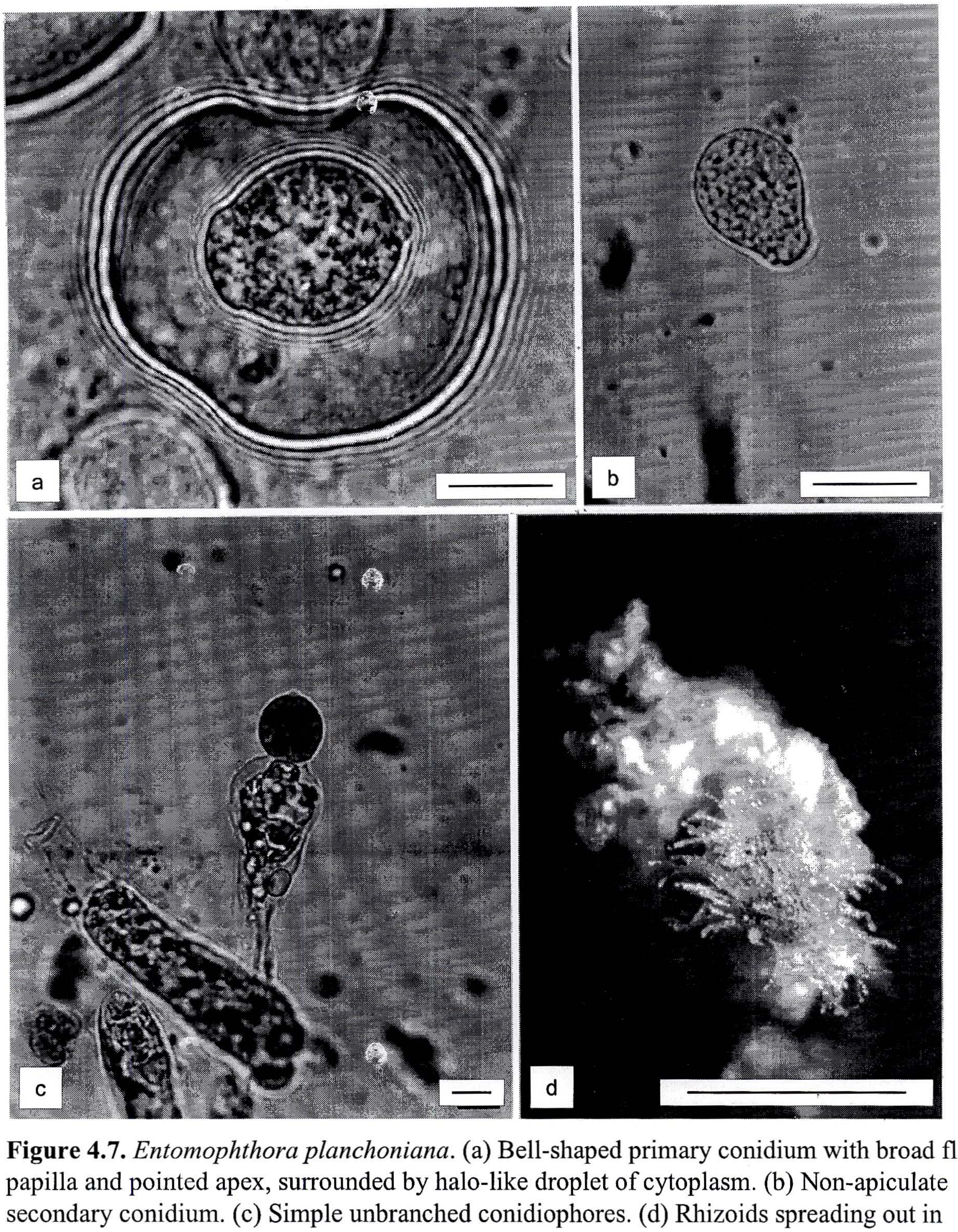

Figure 4.7. *Entomophthora planchoniana.* (a) Bell-shaped primary conidium with broad flat papilla and pointed apex, surrounded by halo-like droplet of cytoplasm. (b) Non-apiculate secondary conidium. (c) Simple unbranched conidiophores. (d) Rhizoids spreading out in different random directions (Bar = 1 mm). Bars = 10 μm.

50); bell-shaped with broad flat papilla and pointed apex (Fig. 4.7a); forcibly discharged. Secondary conidia: budding from the primary conidia; slightly smaller, (12.0)-13.66-(15.0) x (10.0)-11.03-(12.0) µm (n = 50); nonapiculate with more rounded papillae (Fig. 4.7b). Conidiophores: simple unbranched (Fig. 4.7c); generally present only on the dorsal side of the cadaver. Rhizoids: mostly fasciculate, spreading out onto the substrate in different random directions (Fig. 4.7d); almost the same diameter as conidiophores. Diagnosis: primary conidia bell-shaped with flat papilla and pointed apex; 16-22 x 13-19 µm. Secondary conidia: nonapiculate with rounded papilla. Accession nos.: PREM 55865, 55867, 55870 & 55871; ARSEF 199804004, 199804006, 199804008, 199804012 & 199804013.

Verticillium lecanii (Zimmermann) Viégas (Hyphomycetes). Field collection: identified from a single *D. noxia* cadaver collected at Moorreesburg, Western Cape (5 Sept. 1996) (Table 4.1). Cadaver covered by a dense white mycelial growth extending onto the substrate around the cadaver. Isolation: pure cultures established on SDAY and PDA. Description: conidia cylindrical with round apices (Fig. 4.8a), measuring (4.0)-5.26-(7.0) x (1.5)-2.4-(2.5) µm (n = 40); carried apically in slime droplets on conidiogenous cells (Fig. 4.8b); passively discharged. Conidiogenous cells (phialides): awl- to bottle-shaped, usually tapering from the base (Fig. 4.8c); phialides produced in culture strongly verticillioid. Diagnosis: conidia cylindrical with rounded apices; 4-7 x 1-2.5 µm, borne in slime drops on apices of phialides; awl- to bottle-shaped phialides, carried in whorls on hyphae. Accession nos.: PPRI 6692; ARSEF 5706.

Beauveria bassiana (Balsamo) Vuillemin (Hyphomycetes). Field collections: identified from a single *D. noxia* cadaver collected at Langgewens, Western Cape (4 Sept. 1997) (Table 4.1). Cadaver attached to substrate by proboscis. Sporulating cadaver covered with white mycelial growth, later showing powdery appearance. Isolation: pure cultures established on SDAY and PDA. Description: conidia nearly globose (Fig. 4.9a); average diameter 2.03 µm (n = 40); carried singly on conidiogenous cells with flask-like base extending into denticulate rachis (Fig. 4.9b); passively discharged. Diagnosis: denticulate rachis; small globose to subglobose conidia, ≤ 2.5 µm in diameter. Accession nos.: PPRI 6688; ARSEF 5705.

Paecilomyces farinosus (Holm ex S.F. Gray) Brown & Smith (Hyphomycetes). Field collections: no record from field-collected cadavers; collected from a greenhouse colony of *D. noxia* at SGI (Table 4.1). Isolation: cultures established on SDAY and PDA. Description: conidia broad, fusoid to lemon-shaped with more or less tapered ends (Fig. 4.10a), average

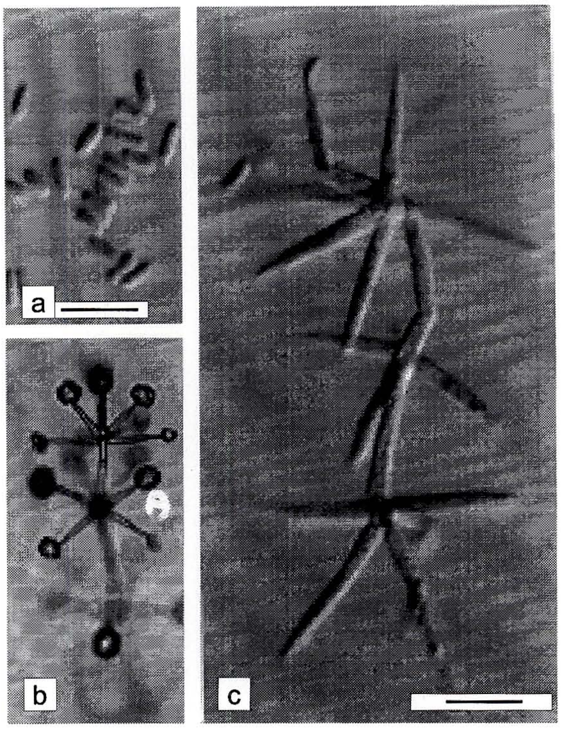

Figure 4.8. *Verticillium lecanii*. (a) Cylindrical conidia with rounded apices. (b) Conidia carried apically in slime droplets on conidiogenous cells. (c) Awl- to bottle-shaped phialides carried in whorls on hyphae. Bars = 10 μm.

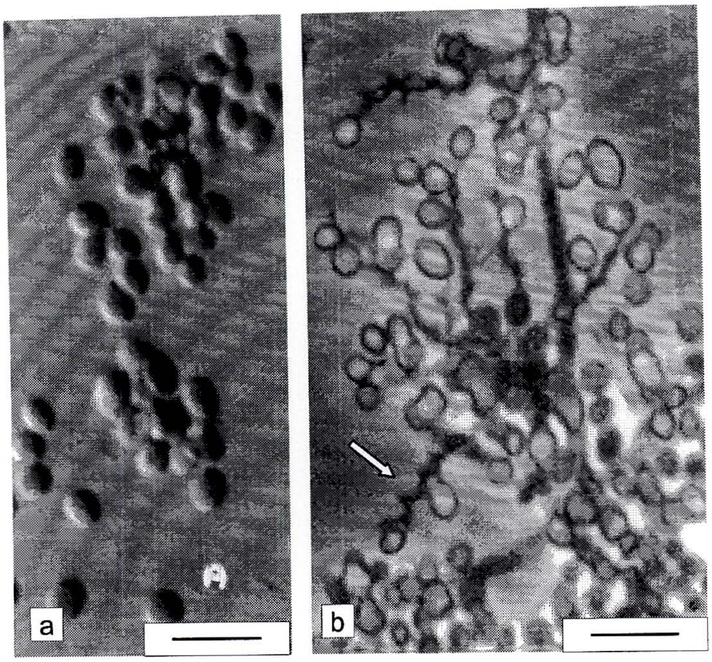

Figure 4.9. *Beauveria bassiana*.(a) Globose to subglobose conidia. (b) Conidia carried singly on conidiogenous cells with flask-like base extending into denticulate rachis (arrow). Bars = 5 μm.

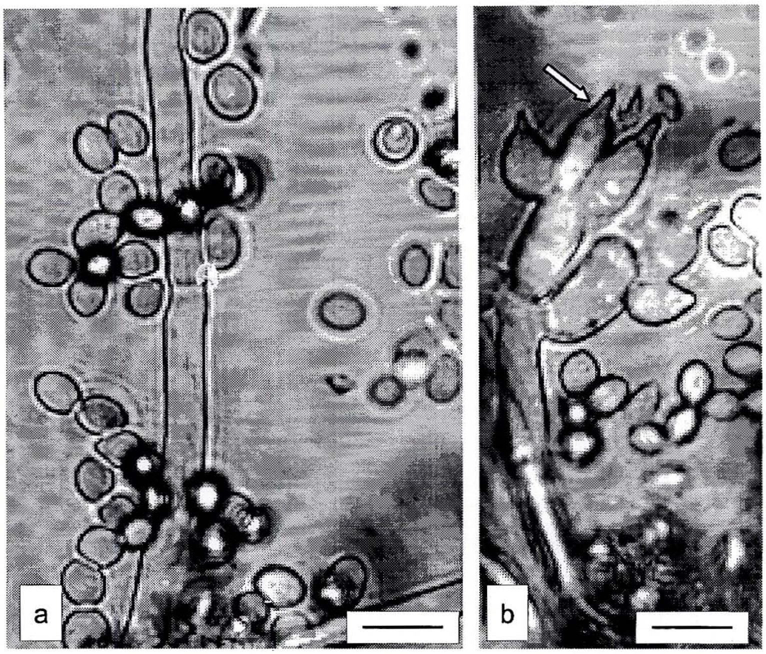

Figure 4.10. *Paecilomyces farinosus*. (a) Conidia roughly lemon-shaped, broad, and with more or less tapered ends. Walls smooth. (b) Phialides with distinct neck (arrow) and swollen (flask-like to globose) base. Bars = 5 μm.

diameter, 3.15 µm (n = 40); basically colourless; carried in long divergent chains on conidiogenous cells; passively discharged. Conidiogenous cells (phialides): distinct neck and swollen (flask-like to globose) base (Fig. 4.10b). Diagnosis: conidia ≤ 3.5 µm in length, roughly lemon-shaped with tapered ends; carried in long, highly divergent conidial chains. Reverse of colony characteristically colourless to pale yellow. Accession nos.: PPRI 6864; ARSEF 6222.

4.4 Discussion

The nine species of entomopathogenic fungi recorded from 1995 to 1999 reflect a great diversity in the indigenous aphid-pathogenic flora from South Africa. Of the six Entomophthorales recorded, four species are considered first reports from this region, *i.e., P. neoaphidis*, *C. thromboides*, *C. obscurus* and *E. planchoniana*. The entomophthoralean *C. coronatus* was previously isolated from the sugar ant *Camponotus* sp. (Hymenoptera: Formicidae) (Schaefer, 1941). *Neozygites fresenii* was reported from *Aphis* sp. in 1911 by Pole-Evans and recently from the cotton aphid, *Aphis gossypii* Glover (Rong & Grobbelaar, 1998). According to Rong & Grobbelaar (1998), the hyphomycete *V. lecanii* was isolated as a parasite of a rust fungus on *Acacia saligna* (Labill.) Wendl. and from the rust *Hemileia vastatrix* Berk. & Broome on *Coffea* sp., with no record of *V. lecanii* in association with an arthropod host. Isolation of *V. lecanii* from an insect host (*D. noxia*) is therefore the first South African report in this regard. On the other hand, *B. bassiana* is known in South Africa from various hosts within the Lepidoptera, Coleoptera, Orthoptera, and Isoptera (Doidge, 1950; Möhr, 1982; Van Rensburg *et al.*, 1988; Hoekstra & Kfir, 1997; Rong & Grobbelaar, 1998; Kfir, 2000), with this observation being the first from a homopteran host. Notably, both *B. bassiana* and *V. lecanii* were rare and occurred only on *D. noxia*. These findings concur with previous findings, as very few of the hundreds of described species in the entomopathogenic Hyphomycetes are specific for aphids, and *B. bassiana* and *V. lecanii* rarely infect aphids under field conditions (Humber, 1991).

Aphid-fungal pathogen associations in South Africa are not well documented, with only seven other reports published in this regard. Doidge (1950) noted the discovery of an entomophthoralean fungus (*Entomophthora aphidis* Hoffman) on an *Aphis* sp. in 1921. However, it can probably be safely assumed that this fungus was identical to what is now known as *P. neoaphidis*. This almost universally distributed and common aphid pathogen was known as *Entomophthora aphidis* from the time of Thaxter's (1888) monograph until the clarification by Remaudière & Hennebert (1980) that *Empusa aphidis* Hoffmann is actually a rare species with an apparently highly localized distribution in Europe. On the other hand, the universally known fungus treated by Thaxter (1888) was given its first formal description as *Erynia neoaphidis* Remaudière & Hennebert and subsequently transferred to the genus *Pandora* Humber (Humber, 1989). Soon after first being reported in South Africa, *D. noxia* was found infected with an unidentified fungus (probably *P. neoaphidis*) during warm moist conditions in the summer-rainfall region (Walters *et al.*, 1980). The hyphomycete *Cladosporium oxysporum* (Bert. & Curt.) was reportedly responsible for epizootics in both *A. gossypii* and *Toxoptera citricida* (Kirkaldy) populations feeding on guava trees (*Psidium guajava* L.) in the Eastern Transvaal (currently the Mpumalanga region) (Samways, 1983; Samways & Grech, 1986). Moreover, isolates of *Fusarium oxysporum* Schlecht., *Cladosporium* sp., *Alternaria* sp., and *Penicillium minioluteum* Dierckx were recorded from *A. gossypii* feeding on *Gossypium hirsitum* L.; some cadavers harbored both *N. fresenii* and a species of *Cladosporium* (Rong & Grobbelaar, 1998). A similar association of *N. fresenii* with *Cladosporium aphidis* Thueman on *Aphis* sp. feeding on *Sorghum bicolor* (L.) Moench was reported by Pole-Evans (1911). Roberts & Humber (1981) and Humber (1991), however, noted that species of *Cladosporium*, *Fusarium* and *Penicillium*, amongst other genera, are often encountered as necrophytic saprobes on aphid cadavers and are, at best, very weak pathogens of aphids. Interestingly, Gustaffson (1965) claimed that insects killed by *Entomophthora fresenii* (= *N. fresenii*) are often found colonized by saprophytic *Cladosporium* sp. Lastly, Von Wechmar *et al.* (1991) reported on an unusual association of aphid-infecting viruses and entomophthoralean fungi (unidentified) occurring in the winter-rainfall region of South Africa.

Most findings of entomopathogenic fungi were from cereal aphids, including seven species from *D. noxia*, but probably this is because small-grain fields were specifically targeted in this study. In both the summer- and winter-rainfall regions, fungi recorded from the cereal-aphid complex were found from early spring through early summer (September through November), coinciding with the second half of the wheat-growth season. Findings from nonagricultural aphid hosts were usually made during late summer through late fall (March through May), with isolated recordings of *E. planchoniana* and *N. fresenii* during the winter months of June and July, respectively (Table 4.1). Noncereal-aphid hosts appeared to play an important role during the wheat off-season (January through May) in sustaining fungal inoculi of at least three entomophthoralean species, *i.e.*, *P. neoaphidis*, *E. planchoniana* and *N. fresenii* (Table 4.1). Resting spores of *C. thromboides*, *C. obscurus* and *N. fresenii* were observed from field-collected cadavers but no resting spores of *P. neoaphidis* or *E. planchoniana* were observed although these two species were the most frequently encountered. Similarly, no resting spores of *P. neoaphidis*, the most prevalent species, were observed during extensive surveys of cereal aphids in the United States (Feng *et al.*, 1990a). No verified (or verifiable) report of resting-spore formation by *P. neoaphidis* has been published by any author about any host or any location worldwide. The only semi-credible report about resting spores being formed by *P. neoaphidis* is that of Uziel & Kenneth (1986), but the physical conditions described are irreproducible. Although not recorded during the winter months of July and August, findings in this study suggest that both *P. neoaphidis* and *E. planchoniana* can successfully infect various aphid hosts during the wheat-off season (Table 4.1).

The impact of fungal mycoses on oversummering populations of *D. noxia* is still unknown, although it will almost certainly be a factor in reducing such populations and therefore possibly the level of infestation of the subsequent crop (Wraight *et al.*, 1993). In the summer-rainfall region of South Africa, the most important alternative host plants of *D. noxia* include volunteer wheat and rescue grass, *Bromus catharticus* Vahl (Hewitt *et al.*, 1984; Aalbersberg *et al.*, 1988a). In addition, four species of grasses, endemic to the winter-rainfall region, have been recently identified as suitable hosts of *D. noxia* (Prinsloo *et al.*, 1997b). Ideally, future surveys should include such alternative hosts to gain a better understanding of the seasonal impact of entomopathogenic fungi on populations of *D. noxia*.

One isolate of *P. neoaphidis* was collected in late summer 1997 from *M. dirhodum* on top of Naude's Nek Pass, where the mean annual temperature is 8-10°C (Schulze, 1997). Follow-up surveys in that area and bordering Lesotho highlands are definitely warranted. Surprisingly, the cosmopolitan species *Zoophthora radicans* (Brefeld) Batko, a major aphid pathogen (Humber, 1991), was not found during these surveys. The occurrence of the fungi collected during the surveys is probably influenced by many factors such as habitat, environment, macro- and microclimate, availability of hosts, etc. The pest status of *D. noxia* can be correlated to the macroclimatic conditions encountered in the various wheat-producing areas of South Africa. In the summer-rainfall region, *i.e.*, central and eastern Free State, infestation during September and October is severe, coinciding with an increase in temperatures and a drop in the available soil-water content (Prinsloo *et al.*, 1995). On the other hand, the Russian wheat aphid is considered a secondary sporadic pest of wheat in the winter-rainfall region of the Western Cape (Prinsloo & Tolmay, 1999b). Because of the Mediterranean climate of this region, moderate temperatures (averaging 15-20°C) and wet conditions (it is not uncommon to find that the plant surfaces remain wet until 11h00 or even noon) prevail during the wheat growth season, a scenario usually conducive to entomophthoralean epizootics (Dean & Wilding, 1971, 1973; Voronina, 1971; Wilding, 1975; Dedryver, 1983; Milner & Bourne, 1983; Wraight *et al.*, 1993; Poprawski & Wraight, 1998). A study conducted by Feng *et al.* (1991a) revealed that, generally, host density rather than relative humidity and temperature adequately explained the occurrence of entomophthoralean mycoses in cereal-aphid populations in the United States. However, during one year of the study, Feng *et al.* (1991a) found that frequent low or moderate rainfall, resulting in prolonged leaf wetness, coincided with effective natural aphid control by fungi. Surveys conducted in the eastern Free State from 1980 through 1992 clearly indicated that Russian wheat aphid populations were generally below average during abnormally wet years in this time period (Dr. F. Du Toit, unpublished data). The impact of insect-pathogenic fungi on both oversummering and crop-infesting populations of Russian wheat aphid and other cereal aphids in South Africa could be more important than was previously thought.

4.5 Current review

Milner (1997) pointed out that five species of aphid-pathogenic fungi dominate under natural conditions in most parts of the world. These species include *P. neoaphidis, E. planchoniana, N. fresenii, Z. radicans,* and *C. obscurus.* The South African surveys revealed much the same tendency although *Z. radicans* was not recorded from any aphid host at any of the 13 sites surveyed. This might be explained by the fact that only 16 aphid species out of a known fauna of 244 species occurring on the African mainland south of the Sahara desert (Millar, 1994) were collected during this study. The spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. *maculata,* is particularly susceptible to *Z. radicans* (Milner, 1997). The closely related yellow clover aphid, *Therioaphis trifolii* (Monell) (Blackman & Eastop, 2000), occurring in South Africa (Annecke & Moran, 1982; Millar, 1994), could be a suitable host for *Z. radicans* and should be surveyed for the presence of this pathogen.

The two Entomophthorales *P. neoaphidis* and *C. obscurus* were the most prevalent species recorded from cereal aphids under field conditions in South Africa (Table 4.1). However, investigation into the comparative impact of these and other fungal species on the cereal-aphid complex clearly underscore the importance of *P. neoaphidis* as natural mortality factor (see also Chapter 5).

The three Hyphomycetes *B. bassiana, P. farinosus* and *V. lecanii*, collected during these surveys, are considered worthy species for development as mycoinsecticides for aphid control (Milner, 1997) and should be further investigated in this regard (see Chapter 6).

CHAPTER 5

PREVALENCES OF FUNGAL PATHOGENS AND OTHER NATURAL ENEMIES OF CEREAL APHIDS (HOMOPTERA: APHIDIDAE) IN WHEAT UNDER DRYLAND AND IRRIGATED CONDITIONS IN SOUTH AFRICA

5.1 Introduction

Since its migration into South Africa in the late 1970's (Walters *et al.*, 1980), the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) (Homoptera: Aphididae), has become established as the principal pest of wheat produced under dryland conditions in the summer rainfall region (Du Toit & Walters, 1984; Aalbersberg *et al.*, 1988a). Pesticides soon became the only effective means of control with more than 13 million Rands spent on insecticides against this aphid during 1986 in the eastern Free State alone (Du Toit, 1988). Moreover, the percentage area planted to wheat in South Africa from 1977 through 1995 dropped by 6% (CIMMYT, 1996). As a net importer of wheat, these statistics clearly underscored the need for improved technologies resulting in higher yields per unit area together with reduced production costs. The implementation, by the Small Grain Institute (SGI) of the South African Agricultural Research Council (ARC), of an integrated-control programme based on host-plant resistance and natural enemies against the Russian wheat aphid (Prinsloo *et al.*, 1995, 1997a) was seen by administrators and farmers as a move in the right direction.

Following studies on the indigenous predators and parasitoids of *D. noxia* (Aalbersberg *et al.*, 1984; Aalbersberg, 1987; Aalbersberg *et al.*, 1988b; Prinsloo, 1990; Prinsloo *et al.*, 1993), four predator and two parasitoid species were imported and released in South Africa as part of a classical biological control initiative (see Chapter 1; Du Toit, 1983; Aalbersberg *et al.*, 1984; Hatting, 1995; Marasas *et al.*, 1997; Prinsloo, 1998; Prinsloo *et al.*, 2002). In contrast, the importance of entomopathogenic fungi as natural mortality factors under South African conditions has received little attention. Only two observations of fungi (unidentified) attacking cereal aphids in the field have been reported from South Africa (Walters *et al.*, 1980; Von Wechmar *et al.*, 1991). However, surveys in the Eurasian endemic habitats of *D. noxia* and in North America and Canada have revealed a great diversity of entomopathogenic fungi infecting this aphid (Carl & Mohyuddin, 1989; Feng *et al.*, 1990a, 1991a; Wraight *et al.*, 1993; Poprawski & Wraight, 1998; Olfert *et al.*, 2001).

In South Africa, wheat is generally grown in three production regions, *i.e.*, winter/spring-planted wheat in the summer-rainfall region (Free State province), winter-planted spring wheat types under dryland conditions within the Mediterranean climate of the Western Cape province, and spring wheat types grown under irrigation in the summer-rainfall region (KwaZulu-Natal province). In a first attempt to investigate the impact on, and identity of epizootic-causing fungi within populations of *D. noxia*, surveys were conducted in the three abovementioned areas during the 1996 and 1997 seasons. Understandably, the locality-specific nature of these surveys would not necessarily reflect the impact throughout all of the macro-climatic production areas surveyed. The data would also provide information on mycoses of other important cereal aphids under dryland conditions, *i.e., Rhopalosiphum padi* (L.) and *Schizaphis graminum* (Rondani) (Rabe *et al*., 1989), and on *Metopolophium dirhodum* (Walker), an aphid preferring irrigated conditions (Annecke & Moran, 1982). Ultimately, these findings will provide baseline information for future use of fungal pathogens in the integrated-control programme presently being developed by ARC-SGI against the Russian wheat aphid and other cereal aphids in South Africa. The species distribution, host range, taxonomy, and accessions of the fungal pathogens collected are discussed in Chapter 4. For completeness, the complex of locally occurring predators and parasitoids active in aphid-infested wheat, was identified to family level.

5.2 Material and methods

None of the fields sampled in the three localities were treated with pesticides prior to the surveys. During all surveys, leaves and/or tillers with their resident cadavers or mummies were placed in plastic zip-lock bags (170mm x 170mm) and promptly transported to the laboratory for processing (within 24 hours). Aphids were identified according to the keys of Prior (1974) and Du Toit & Aalbersberg (1980). Cadavers showing external signs of mycosis (rhizoids, cystidia, mycelia and spores) were mounted in a drop of lactophenol using 2% aceto-carmine as stain and the fungi were identified to species level (see Chapter 4). Aphids parasitized by parasitoids belonging to the Aphidiinae could be distinguished by a light brown tanned aphid cuticle with abdominal walls greatly distended by internal parasitoid cocoon before adult parasitoid emergence. Similarly, aphids parasitized by parasitoids belonging to the Aphelinidae exhibited a black tanned cuticle with an internal cocoon not distending the abdominal wall. Dividing the number of aphids killed by fungi or parasitoids by the number of dead plus living aphids derived estimates of pathogen and parasitoid prevalences. Only cadavers with active (sporulating) fungus were included in the calculations. Chi-square analysis was used for comparisons of binomial (proportion infection or parasitism) data (Snedecor & Cochran, 1967).

5.2.1 Irrigated wheat, summer rainfall (Bergville/Winterton; KwaZulu-Natal Province)

Surveys were conducted on the farms 'The Bend' (Bergville area; S28°45.632'/E29°18.091') and 'Erasmus Dam' (Winterton area; S28°48.168'/E29°31.390'). Wheat ('Marico') was produced under centre-pivot irrigation at both sites. Both farms were surveyed on 22 and 29 October 1996. The Bergville farm was further surveyed on 17 and 25 September, and on 1 (*i.e.*, growth stage 65 according to Tottman, 1987), 8 and 22 October 1997. On each sampling date, one tiller (sample unit) was collected at random (without regard to evidence of aphid feeding) at each of 10 equally spaced points 2 metres apart along five parallel transects (5 m apart) within the field, totaling 50 tillers. Transects were not permanently marked and distances were measured by pacing. Therefore, although located in the same area, tillers were never sampled at the same point locations. To provide additional data, 100 live *M. dirhodum* were randomly collected on 22 October 1996 from both the Winterton and Bergville sites. On 25 September and 1 and 8 October 1997, respectively 120, 150 and 48 live *M. dirhodum* were collected from the Bergville site. For live aphid collection, leaves and/or tillers with residing aphids were picked and placed in brown paper-bags; all bags (150mm x 300mm) were transported inside a cooler box with one or two freezer blocks. These aphids were maintained in the laboratory for identification of fungi and/or parasitoids which emerged from parasitized aphids. The aphids were placed on wheat seedlings (cultivar 'Tugela'; 3-leaf stage) growing in 2-litre pots. No more than 50 aphids were placed on each seedling and the seedling was caged with a plastic 2-litre soft drink bottle containing two large (50 mm x 50 mm) vents covered with nylon sheer. The units were kept in the laboratory at room temperature and inspected daily for 2 days for aphid mortality.

5.2.2 Dryland wheat, winter rainfall (Langgewens; Western Cape Province)

Dryland wheat (cultivar 'Kariega', growth stage 41) was sampled at the Langgewens Experimental Station (S33°16.329'/E18°42.484') on 5 September 1997. Sixty-nine flag leaves (sample units) showing typical Russian wheat aphid feeding damage (see Chapter 1 section 1.2), were randomly collected from a 0.5 ha (50 m x 100 m) wheat field. Due to the high level of aphid-infestation, only flag leaves were sampled instead of tillers. Sample units collected were at least 3 m apart.

5.2.3 Dryland wheat, summer rainfall (Bethlehem; Free State Province)

Surveys were conducted at ARC-SGI during both 1996 (5, 14 and 20 November; site S28°09.730'/E28°17.848') and 1997 (3 November; site 28°09.756'/E28°17.787'). In both years the wheat (cultivar 'Tugela') plots sampled did not exceed 300 m^2 in size. A single flag leaf was the sample unit. Only leaves showing typical Russian wheat aphid feeding damage (*i.e.*, rolled-up leaves with streaking; see Chapter 1 section 1.2) were collected. The number of flag leaves collected on each sampling date is given in Table 5.1. All aphids on these leaves were sampled. Aphid numbers sampled are given in Table 5.2.

To provide additional data, 100 live *D. noxia* and 37 *R. padi* were randomly collected on 20 November 1996 and reared in the laboratory (as described above) for identification of fungi and/or parasitoids which emerged from parasitized aphids. Tillers from which these aphids were collected were chosen without regard to evidence of aphid feeding (*i.e.*, random fashion).

5.3 Results

5.3.1 Aphid populations

Diuraphis noxia was the most abundant aphid species under dryland conditions (summer-rainfall) at the Bethlehem site during both 1996 and 1997, with up to 238 *D. noxia* recorded per flag leaf (14 November 1996; data not shown). Populations of *D. noxia* did not fluctuate significantly over the sampling period and their numbers remained high. The highest mean number of *D. noxia* recorded per flag leaf was 99.2 on 14 November 1996 (Table 5.1). *Rhopalosiphum padi* was the only other cereal aphid recorded in substantial numbers. Although recorded during both years, *M. dirhodum* was present in low numbers. *Schizaphis graminum* was recorded only during 1996 averaging 0.4 aphids per flag leaf (20 November) (Table 5.1).

Under dryland conditions at Langgewens (winter-rainfall region of the Western Cape), the most abundant aphid was *R. padi*, averaging 57.2 aphids per flag leaf (Table 5.1.).

Table 5.1. Infestation levels and population densities of cereal aphids infesting dryland and irrigated wheat in South Africa during 1996-1997

Crop sample date (Field site)	Number of SU[a]	% SU infested with aphids	Mean number of aphids per SU ± SEM	% SU (mean number of aphids per sample unit ± SEM) infested with					
				D. noxia	*R. padi*	*M. dirhodum*	*S. avenae*	*R. maidis*	*S. graminum*
Dryland wheat									
5 Nov. 1996 (Bethlehem)	7	100	87.4 ± 22.7	100 (81.7 ± 24.0)	42.9 (5.7 ± 3.6)	0.0	0.0	0.0	0.0
14 Nov. 1996 (Bethlehem)	10	100	113.0 ± 25.5	100 (99.2 ± 21.7)	80.0 (13.8 ± 5.5)	0.0	0.0	0.0	0.0
20 Nov. 1996 (Bethlehem)	40	100	61.6 ± 7.4	100 (51.8 ± 6.4)	80.0 (9.1 ± 1.7)	5.0 (0.2 ± 0.1)	0.0	0.0	10 (0.4 ± 0.2)
3 Nov. 1997 (Bethlehem)	50	100	100.6 ± 8.5	100 (76.6 ± 5.9)	64.0 (21.8 ± 5.2)	22.0 (1.1 ± 0.5)	0.0	0.0	0.0
5 Sept. 1997 (Langgewens)	69	100	64.0[b]	79.7 (6.5)[b]	100 (57.2)[b]	5.8 (0.1 ± 0.1)	17.4 (0.3 ± 0.1)	4.3 (0.1 ± 0.1)	0.0
Irrigated wheat									
22 Oct. 1996 (Bergville)	50	82.0	5.1 ± 1.1	0.0	30.0 (1.0 ± 0.4)	66.0 (4.0 ± 1.0)	0.0	0.0	0.0

Table 5.1. Infestation levels and population densities of cereal aphids infesting dryland and irrigated wheat in South Africa during 1996-1997 (continued)

Crop sample date (Field site)	Number of SU[a]	% SU infested with aphids	Mean number of aphids per SU ± SEM	% SU (mean number of aphids per sample unit ± SEM) infested with					
				D. noxia	*R. padi*	*M. dirhodum*	*S. avenae*	*R. maidis*	*S. graminum*
22 Oct. 1996 (Winterton)	50	100	14.2 ± 2.3	0.0	58.0 (2.9 ± 0.8)	98.0 (10.8 ± 1.7)	8.0 (0.1 ± 0.1)	16.0 (0.3 ± 0.2)	0.0
29 Oct. 1996 (Bergville)	50	52.0	3.2 ± 1.0	0.0	14.0 (0.5 ± 0.4)	40.0 (2.4 ± 0.7)	10.0 (0.2 ± 0.1)	0.0	0.0
29 Oct. 1996 (Winterton)	50	44.0	1.4 ± 0.3	0.0	0.0	44.0 (1.4 ± 0.3)	0.0	0.0	0.0
17 Sept. 1997 (Bergville)	50	62.0	1.9 ± 0.4	0.0	50.0 (1.1 ± 0.3)	14.0 (0.5 ± 0.3)	6.0 (0.2 ± 0.2)	2.0 (0.02 ± 0.02)	0.0
25 Sept. 1997 (Bergville)	50	60.0	2.3 ± 0.5	0.0	36.0 (1.2 ± 0.5)	34.0 (1.0 ± 0.3)	4.0 (0.2 ± 0.2)	0.0	0.0
1 Oct. 1997 (Bergville)	50	48.0	1.5 ± 0.6	0.0	28.0 (0.5 ± 0.1)	26.0 (0.9 ± 0.5)	4.0 (0.1 ± 0.1)	0.0	0.0
8 Oct. 1997 (Bergville)	50	40.0	0.9 ± 0.3	0.0	8.0 (0.2 ± 0.1)	32.0 (0.7 ± 0.2)	4.0 (0.04 ± 0.03)	0.0	0.0
22 Oct. 1997 (Bergville)	50	12.0	0.2 ± 0.1	0.0	0.0	10.0 (0.2 ± 0.1)	2.0 (0.02 ± 0.02)	0.0	0.0

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[a] The sample unit (SU) was one tiller at Winterton and Bergville and one flag leaf at other sites.

[b] SEM was not calculated as some aphids left the flag leaves and became intermixed in the collection bag.

Diuraphis noxia was well established throughout the field with 79.7% flag leaves infested. Their density, averaging 6.5 aphids per flag leaf, was however considerably lower when compared with *R. padi*. Three other aphid species, *M. dirhodum*, *Rhopalosiphum maidis* (Fitch), and *Sitobion avenae* (F.) were also recorded though in very low numbers (Table 5.1).

Under irrigated conditions in the Winterton/Bergville area (summer rainfall region), *M. dirhodum* dominated during 1996, followed by *R. padi*, *S. avenae* and *R. maidis*. During the following year *R. padi* dominated during the second half of September but had disappeared by 22 October while 10% of tillers were still found infested with *M. dirhodum*. *Sitobion avenae* occurred sporadically throughout 1997 with only a single finding of *R. maidis* on 17 September (Table 5.1). Notably, no *D. noxia* were recorded under irrigated conditions in either 1996 or 1997.

5.3.2 Hymenopteran parasitoids

During 1997, the highest total aphid parasitism was observed under irrigated conditions at the Bergville site (Table 5.2). The most parasitised species was *R. padi*. Parasitism of this species increased significantly from 5.1% on 25 September, to 25.0% on 1 October 1997 ($\chi^2 = 5.09$; df = 1; $P = 0.023$; $n = 83$ aphids). All mummies were of the Aphidiinae. Similarly, from the 120 and 150 live *M. dirhodum* collected on 25 and 1 October, respectively, > 93% of mummies obtained from these aphids in the laboratory were of the Aphidiinae (Table 5.3). In contrast, no parasitism was recorded on any cereal aphid at either the Winterton or Bergville sites during 1996. Although at a low level, highest parasitism (1.3%; Aphidiinae) was recorded from *R. padi* at Langgewens on 5 September 1997 (Table 5.2). During the latter survey no *D. noxia* mummies were recorded despite the fact that this aphid was present on 87.5% of flag leaves harbouring *R. padi* mummies. Parasitism of *D. noxia* did, however, occur under dryland conditions in the summer rainfall region (Bethlehem) but did not exceed 0.4 and 0.3% in 1996 and 1997, respectively. Although the first mummy observed at Bethlehem during 1996 (14 November) was of the Aphidiinae, parasitism 6 days later was predominantly (> 75%) due to aphelinid-type wasps. In contrast, > 80% of mummified *D. noxia* collected on 3 November 1997 were of the Aphidiinae (Table 5.2).

Table 5.2 . Parasitoids in populations of cereal aphids infesting dryland and irrigated wheat in South Africa during 1996-1997

Crop sample date (Field site)	% of SU[a] with parasitized cereal aphids	Mean number of parasitized aphids per SU	% parasitism (number of aphids in sample in parentheses) of						% parasitism of all cereal aphids
			D. noxia	*R. padi*	*M. dirhodum*	*S. avenae*	*R. maidis*	*S. graminum*	
Dryland wheat									
5 Nov 1996 (Bethlehem)	0.0 (7)[b]	0.0	0.0 (572)	0.0 (40)	- (0)	- (0)	- (0)	- (0)	0.0 (612)
14 Nov 1996 (Bethlehem)	10.0 (10)	0.1	0.1 (992)	0.0 (138)	- (0)	- (0)	- (0)	- (0)	0.1 (1130)
20 Nov 1996 (Bethlehem)	15.0 (40)	0.2	0.4 (2078)	0.0 (365)	0.0 (7)	- (0)	- (0)	0.0 (15)	0.4 (2465)
3 Nov 1997 (Bethlehem)	22 (50)	0.3	0.3 (3886)	0.3 (1092)	0.0 (54)	- (0)	- (0)	- (0)	0.3 (5032)
5 Sept 1997 (Langgewens)	46.4 (69)	0.7	0.0 (446)	1.3 (3945)	0.0 (6)	0.0 (19)	0.0 (7)	- (0)	1.1 (4423)
Irrigated wheat									
22 Oct 1996 (Bergville)	0.0 (41)	0.0	- (0)	0.0 (52)	0.0 (202)	- (0)	- (0)	- (0)	0.0 (254)

Table 5.2. Parasitoids in populations of cereal aphids infesting dryland and irrigated wheat in South Africa during 1996-1997 (continued)

Crop sample date (Field site)	% of SU[a] with parasitized cereal aphids	Mean number of parasitized aphids per SU	% parasitism (number of aphids in sample in parentheses) of						% parasitism of all cereal aphids
			D. noxia	*R. padi*	*M. dirhodum*	*S. avenae*	*R. maidis*	*S. graminum*	
22 Oct. 1996 (Winterton)	0.0 (50)	0.0	- (0)	0.0 (147)	0.0 (540)	0.0 (5)	0.0 (17)	- (0)	0.0 (709)
29 Oct 1996 (Bergville)	0.0 (26)	0.0	- (0)	0.0 (27)	0.0 (122)	0.0 (10)	- (0)	- (0)	0.0 (159)
29 Oct 1996 (Winterton)	0.0 (22)	0.0	- (0)	- (0)	0.0 (70)	- (0)	- (0)	- (0)	0.0 (70)
17 Sept 1997 (Bergville)	0.0 (31)	0.0	- (0)	0.0 (56)	0.0 (26)	0.0 (10)	0.0 (1)	- (0)	0.0 (93)
25 Sept 1997 (Bergville)	6.7 (30)	0.1	- (0)	5.1 (59)	0.0 (49)	0.0 (9)	- (0)	- (0)	2.6 (117)
1 Oct. 1997 (Bergville)	29.2 (24)	0.3	- (0)	25.0 (24)	0.0 (45)	25.0 (4)	- (0)	- (0)	9.6 (73)
8 Oct 1997 (Bergville)	10.0 (20)	0.1	- (0)	0.0 (9)	5.9 (34)	0.0 (2)	- (0)	- (0)	4.4 (45)
22 Oct. 1997 (Bergville)	16.7 (6)	0.2	- (0)	- (0)	12.5 (8)	0.0 (1)	- (0)	- (0)	11.1 (9)

[a] Sample units (aphid infested)

[b] Number of infested sample units examined in parentheses

Table 5.3. Latent fungal infection and parasitism in cereal aphids field-collected live and reared in the laboratory

Crop sample date (Field site)	Aphid	Number of aphids reared	Number of mycoses / mummies obtained			
			P. neoaphidis	*C. obscurus*	Aphidiinae	Aphelinidae
20 Nov 1996 (Bethlehem)	*D. noxia*	100	38	0	0	0
	R. padi	37	2	0	0	0
22 Oct 1996 (Winterton)	*M. dirhodum*	100	14	2	0	0
22 Oct 1996 (Bergville)	*M. dirhodum*	100	11	0	0	0
25 Sept 1997 (Bergville)	*M. dirhodum*	120	13	31	9	0
1 Oct 1997 (Bergville)	*M. dirhodum*	150	13	5	6	1
8 Oct 1997 (Bergville)	*M. dirhodum*	48	5	4	4	0

5.3.3 Predators

Predator numbers remained low throughout all surveys with only syrphid larvae and coccinellids recorded. At the time of initiation of sampling at Bethlehem on 5 November 1996, one syrphid egg and five coccinellid eggs were observed. However, the only actively feeding predator observed was one syrphid larva on 20 November 1996. Similarly, a single syrphid larva was the only predator recorded at Bethlehem on 3 November 1997. The highest numbers of predators under irrigated conditions during 1996 were recorded at the Winterton site on 22 October. On this date, syrphid larvae were found on 4% of tillers sampled and their overall density was 0.04 larva per tiller. The only sign of other predatory insects present during 1996 was a coccinellid pupa found at Bergville on 29 October. Predator incidence during 1997 followed much the same pattern as during the previous season. The highest numbers recorded (Bergville) were on 25 September when syrphid larvae were found on 4% of tillers sampled and their overall density was 0.04 larva per tiller. One coccinellid larva and adult [*Hippodamia variegata* (Goeze)] were found on 17 September and 22 October, respectively. No predator activity was observed on either 1 or 8 October of that year.

5.3.4 Fungal pathogens

5.3.4.1 Irrigated wheat, summer rainfall (Bergville/Winterton)

During 1996, the highest fungal parasitism at the Bergville site (22.4% of the total cereal aphid population) was recorded on 22 October (Table 5.4). This level, however, declined significantly to 11.9% on 29 October (χ^2 = 6.49; df = 1; P = 0.011; n = 413 aphids). The highest incidence recorded per aphid species was 28.2% infection of *M. dirhodum* (22 October). A single fungus-killed *S. avenae* was collected on 29 October, with no fungal parasitism recorded on *R. padi* on either date (Table 5.4). Notably, the entomophthoralean fungus, *Pandora neoaphidis* (Remaudière and Hennebert) Humber, was the only pathogen recorded from field-collected cadavers during 1996 (Table 5.5). Similarly, 100 live *M. dirhodum* randomly collected on 22 October at Bergville and reared in the laboratory for 48 hours, yielded 11 cadavers, all infected by *P. neoaphidis* (Table 5.3).

Fungal infection recorded at the Winterton site increased significantly from 17.1% on 22 October to 77.1% of the total aphid population on 29 October (χ^2 = 128.59; df = 1; P < 0.001; n = 779 aphids) (Table 5.4). However, migration of aphids due to maturing of the crop

Table 5.4. Entomopathogenic fungi in populations of cereal aphids infesting dryland and irrigated wheat in South Africa during 1996-1997

Crop sample date (Field site)	% of SU[a] with infected cereal aphids	Mean number of infected aphids per SU	% infection (number of aphids in sample in parentheses) of						% infection (number of aphids in sample in parentheses) of all cereal aphids
			D. noxia	*R. padi*	*M. dirhodum*	*S. avenae*	*R. maidis*	*S. graminum*	
Dryland wheat									
5 Nov. 1996 (Bethlehem)	85.7 (7)[b]	5.1	6.3 (572)	0.0 (40)	- (0)	- (0)	- (0)	- (0)	5.9 (612)
14 Nov. 1996 (Bethlehem)	100 (10)	49.6	50.0 (992)	0.0 (138)	- (0)	- (0)	- (0)	- (0)	43.9 (1130)
20 Nov. 1996 (Bethlehem)	95.0 (40)	7.3	14.1 (2078)	0.3 (365)	0.0 (7)	- (0)	- (0)	0.0 (15)	11.9 (2465)
3 Nov. 1997 (Bethlehem)	82.0 (50)	14.6	18.6 (3886)	1.0 (1092)	0.0 (54)	- (0)	- (0)	- (0)	4.5 (5032)
5 Sept. 1997 (Langgewens)	81.2 (69)	2.9	14.3 (446)	4.4 (3945)	0.0 (6)	0.0 (19)	0.0 (7)	- (0)	4.3 (4423)
Irrigated wheat									
22 Oct. 1996 (Bergville)	43.9 (41)	1.4	- (0)	0.0 (52)	28.2 (202)	- (0)	- (0)	- (0)	22.4 (254)

Table 5.4. Entomopathogenic fungi in populations of cereal aphids infesting dryland and irrigated wheat in South Africa during 1996-1997 (continued)

Crop sample date (Field site)	% of SU[a] with infected cereal aphids	Mean number of infected aphids per SU	% infection (number of aphids in sample in parentheses) of						% infection (number of aphids in sample in parentheses) of all cereal aphids
			D. noxia	*R. padi*	*M. dirhodum*	*S. avenae*	*R. maidis*	*S. graminum*	
22 Oct. 1996 (Winterton)	72.0 (50)[b]	2.4	- (0)	0.0 (147)	21.9 (540)	0.0 (5)	17.6 (17)	- (0)	17.1 (709)
29 Oct. 1996 (Bergville)	42.3 (26)	0.7	- (0)	0.0 (27)	14.8 (122)	10 (10)	- (0)	- (0)	11.9 (159)
29 Oct. 1996 (Winterton)	95.5 (22)	2.5	- (0)	- (0)	77.1 (70)	- (0)	- (0)	- (0)	77.1 (70)
17 Sept. 1997 (Bergville)	3.2 (31)	0.03	- (0)	0.0 (56)	0.0 (26)	10 (10)	0.0 (1)	- (0)	1.1 (93)
25 Sept. 1997 (Bergville)	6.7 (30)	0.1	- (0)	1.7 (59)	2.0 (49)	0.0 (9)	- (0)	- (0)	1.7 (117)
1 Oct. 1997 (Bergville)	16.7 (24)	0.3	- (0)	0.0 (24)	13.3 (45)	25.0 (4)	- (0)	- (0)	9.6 (73)
8 Oct. 1997 (Bergville)	30 (20)	0.4	- (0)	0.0 (9)	20.6 (34)	0.0 (2)	- (0)	- (0)	15.6 (45)
22 Oct. 1997 (Bergville)	33.3 (6)	0.3	- (0)	- (0)	25.0 (8)	0.0 (1)	- (0)	- (0)	22.2 (9)

[a] Sample units (aphid infested).

[b] Number of infested sample units examined in parentheses.

Table 5.5. Identification of entomopathogenic fungi on cereal aphid cadavers collected from dryland and irrigated wheat in South Africa during 1996-1997

Crop sample (Field site)	Aphid species	Number of cadavers examined	Number of aphids infected with				
			P. neoaphidis	*C. obscurus*	*C. thromboides*	*E. planchoniana*	*B. bassiana*
Dryland wheat							
5 Nov. 1996 (Bethlehem)	*D. noxia*	20	20	-	-	-	-
14 Nov. 1996 (Bethlehem)	*D. noxia*	150	150	-	-	-	-
20 Nov. 1996 (Bethlehem)	*D. noxia*	145	145	-	-	-	-
	R. padi	1	1	-	-	-	-
3 Nov. 1997 (Bethlehem)	*D. noxia*	721	716	-	-	5	-
	R. padi	11	11	-	-	-	-
5 Sept. 1997 (Langgewens)	*D. noxia*	64	63	-	-	-	1
	R. padi	135	132	2	1	-	-

Table 5.5. Identification of entomopathogenic fungi on cereal aphid cadavers collected from dryland and irrigated wheat in South Africa during 1996-1997 (continued)

Crop sample (Field site)	Aphid species	Number of cadavers examined	Number of aphids infected with				
			P. neoaphidis	*C. obscurus*	*C. thromboides*	*E. planchoniana*	*B. bassiana*
Irrigated wheat							
22 Oct. 1996 (Bergville)	*M. dirhodum*	25	25	-	-	-	-
29 Oct. 1996 (Bergville)	*M. dirhodum*	10	10	-	-	-	-
	S. avenae	1	1	-	-	-	-
17 Sept. 1997 (Bergville)	*S. avenae*	1	1	-	-	-	-
25 Sept. 1997 (Bergville)	*M. dirhodum*	1	-	1	-	-	-
	R. padi	1	-	1	-	-	-
1 Oct. 1997 (Bergville)	*M. dirhodum*	6	6	-	-	-	-
	S. avenae	1	1	-	-	-	-
8 Oct. 1997 (Bergville)	*M. dirhodum*	7	6	1	-	-	-
22 Oct. 1997 (Bergville)	*M. dirhodum*	2	2	-	-	-	-
22 Oct. 1996 (Winterton)	*M. dirhodum*	60	60	-	-	-	-
	R. maidis	3	3	-	-	-	-
29 Oct. 1996 (Winterton)	*M. dirhodum*	25	25	-	-	-	-

had a significant impact on the aphid population as the mean number of mycosed plus non-mycosed aphids per tiller declined from 14.2 to 1.4 on 22 and 29 October, respectively (t = 9.73; df = 51; $P < 0.001$; n = 100) (Table 5.1). The high level of infection recorded on 29 October may be attributed to the fact that cadavers were affixed to the plants by fungal rhizoids (see Chapter 4), thus leading to an overestimate of infection. For this reason surveys were terminated on 29 October. As at Bergville, *M. dirhodum* was the most infected species (21.9% infection on 22 October) at Winterton, with no infection recorded on *R. padi*. Once again, *P. neoaphidis* was the only species identified from > 50% of cadavers collected during 1996 (Table 5.5). From 100 live *M. dirhodum* collected on 22 October and reared in the laboratory for 48 hours, 14.0% were killed by *P. neoaphidis* and 2.0% by a second entomophthoralean, *Conidiobolus obscurus* (Hall and Dunn) Remaudière and Keller (Table 5.3).

The first sign of fungal activity at the Bergville site during 1997 was observed on 17 September following the collection of a single *S. avenae* cadaver infected by *P. neoaphidis* (Table 5.4 and 5.5). Additional scouting outside the sample area revealed two *P. neoaphidis*-infected cadavers (*M. dirhodum* and *R. padi*) and one *S. avenae* cadaver infected by *C. obscurus* (data not shown). In comparison with 1996, a slightly lower level of infection (due to *P. neoaphidis*; Table 5.5) was recorded from *M. dirhodum* increasing, although not significantly, from 13.3% on 1 October to peak at 20.6% 7 days later ($\chi^2 = 0.31$; df = 1; P = 0.508; n = 79 aphids). Although even higher infection was recorded on 22 October (25.0%), this increase was not significant ($\chi^2 = 0.042$; df = 1; P = 0.819; n = 42 aphids) and considered an overestimate of infection due to migration of aphids as was the case at Bergville on 29 October 1996. Infection of *R. padi* did not exceed 1.7% (25 September) (Table 5.4). The number of tillers infested with *S. avenae* varied from 2 to 4% throughout all survey dates yielding only one *P. neoaphidis*-infected cadaver on each of 17 September and 1 October (Table 5.5).

5.3.4.2 Dryland wheat, winter rainfall (Langgewens)

A total of 4 423 aphids were collected from the sample units (Table 5.2). The highest fungal infection (14.3%) was recorded from *D. noxia* as opposed to < 5% recorded from *R. padi* (Table 5.4), despite > 90% of the *R. padi* population occupying the same feeding niche as *D. noxia* (inside rolled-up leaves). It was thus exposed to the same microclimatic conditions supporting mycoses in *D. noxia*. Comparing individual flag leaves, the highest prevalence of mycosis per aphid species was 17.0 and 100% for *R. padi* and *D. noxia* respectively (data not shown), again suggesting some level of low susceptibility in *R. padi*. Although *M. dirhodum*, *S. avenae,* and *R. maidis* were present in low numbers on the flag leaves no mycosed individuals were recorded from these species (Table 5.4). *Pandora neoaphidis* was the predominant fungal species, recorded from 98.0% of aphid cadavers (Table 5.5). Two other entomophthoralean species, *C. obscurus* and *Conidiobolus thromboides* Drechsler, and the hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin, were identified from the remaining 2.0% of cadavers collected (Table 5.5).

5.3.4.3 Dryland wheat, summer rainfall (Bethlehem)

The first sample of the survey, collected 5 November 1996, indicated that at least one species of fungus was active within the *D. noxia* population, although at a low level (Table 5.4). However, fungal infection of *D. noxia* increased significantly from 6.3% on 5 November to 50.0% of the population on 14 November ($\chi^2 = 306.83$; df = 1; $P < 0.001$; $n = 1\ 564$ aphids). Over the next 6 days this level decreased significantly to 14.1% ($\chi^2 = 452.90$; df = 1; $P < 0.001$; $n = 3\ 070$ aphids). All of 315 randomly selected *D. noxia* cadavers (1996) were found infected by *P. neoaphidis* (Table 5.5). Although occurring within the same rolled-up flag leaves as *D. noxia*, *R. padi* appeared virtually free of fungal infection with only a single *R. padi* cadaver (out of 365 aphids collected on 20 November) infected by *P. neoaphidis* (Table 5.5). An additional 100 live *D. noxia* and 37 live *R. padi*, randomly collected on 20 November and reared in the laboratory, yielded 38 *D. noxia* and 2 *R. padi* cadavers, all infected by *P. neoaphidis* (Table 5.3). Similarly, fungal infection of *D. noxia* recorded on 3 November 1997 was significantly higher than that recorded on *R. padi* on the same date (18.6% versus 1.0%; $\chi^2 = 207.855$; df = 1; $P < 0.001$; $n = 4\ 978$ aphids). No mycosed *M. dirhodum* were collected. This was attributed to the fact that most *M. dirhodum* were found feeding outside the rolled leaves, thus minimizing the chances of coming into contact with fungal inoculum (sporulating *D. noxia* cadavers) present inside the leaves. Clearly, *P. neoaphidis* was the epizootic-causing agent, with only one other entomophthoralean recorded, *i.e.*, *Entomophthora planchoniana* Cornu, on *D. noxia* (Table 5.5).

5.4 Discussion

5.4.1 Irrigated wheat, summer rainfall (Bergville/Winterton)

Both *P. neoaphidis* and *C. obscurus* recorded under irrigated conditions in the Bergville/Winterton region also rated amongst the most important species infecting cereal aphids on irrigated spring-planted wheat in southwestern Idaho (Feng *et al.*, 1990a, 1991a, 1992b) and Wellington, Colorado (Wraight *et al.*, 1993). During this study *M. dirhodum* clearly exhibited high susceptibility toward *P. neoaphidis* as infection rates of 22-77% were recorded at the Winterton site (Table 5.4). However, during 1997 the prevalences of fungus-induced mortalities in aphids reared from live field-collected aphids tended to differ from that based on subsequent field counts. From 120 field-collected (on 25 September 1997) and subsequently laboratory-reared *M. dirhodum*, only 10.8% were found infected by *P. neoaphidis* as opposed to 25.8% infected by *C. obscurus* (Table 5.3). Therefore, assuming that *C. obscurus* was well established within the *M. dirhodum* population, the 0% occurrence of this pathogen species compared with that of *P. neoaphidis* (100% of cadavers) recorded in the field 6 days later (Table 5.5) was surprising. The relatively secure mode of attachment in *P. neoaphidis*-infected cadavers (*i.e.*, discoid-like holdfasts) versus the weaker proboscis-attached cadavers infected by *C. obscurus* (see Chapter 4), may have been an important contributing factor in retaining more successfully *P. neoaphidis*-infected cadavers on the substrate, especially under overhead-irrigated conditions. Moreover, according to data generated over a three-year period by Feng *et al.* (1990a), significantly fewer field-collected *M. dirhodum* cadavers were found killed by *Conidiobolus* spp. than cadavers obtained in the laboratory from field-collected live *M. dirhodum* (6.9% versus 26.2%), providing further evidence for the postulate stated above. Although not significantly different, the percentage of laboratory-reared *M. dirhodum* infected by *C. obscurus* was consistently higher than the percentage of aphid cadavers simultaneously observed infected by this fungus during field counts, *i.e.*, on 1 October (27.8% reared versus 0% field) and 8 October (44.4% reared versus 14.3% field) (Tables 5.3 and 5.5). These observations suggest that estimates of infection based on direct cadaver counts may not reflect the true level of disease induced by a fungus; at best, this method gives only a crude estimate.

The inherent susceptibility of *M. dirhodum* to fungi, particularly to *P. neoaphidis*, has not only been demonstrated in laboratory bioassays (Feng & Johnson, 1991), but also under field conditions (Dean & Wilding, 1973; Feng *et al.*, 1990a, 1991a), largely due to the favourable microclimatic niche (*i.e.*, on the ventral surface of lower leaves) typically occupied by this aphid species on the wheat plant (Feng *et al.*, 1991a). Moreover, under irrigated conditions in the Winterton/Bergville area, wheat is planted at a density of *ca.* 120 kg/ha (Ybema & Purchase, 1999), resulting in a dense crop canopy, which should protect fungal inocula from wind and ultraviolet radiation. However, despite these seemingly favourable conditions, the level of infection recorded from *R. padi* did not exceed 1.7% (25 September 1997), with no *R. padi* cadavers recorded during the three subsequent survey dates. This observation was made despite an increase in *P. neoaphidis* inocula developing within the *M. dirhodum* population (Table 5.4), seemingly increasing the likelihood of *R. padi* coming into contact with sporulating cadavers and/or actively dispersed ballistospores. Similarly, only 0.28% fungal infection was reported from *R. padi* attacking irrigated corn in southwestern Idaho despite favourable numbers of hosts and apparently suitable environmental conditions (Feng *et al.*, 1990a). These observations suggest that *R. padi* may be less susceptible than *M. dirhodum* to *P. neoaphidis*. This hypothesis is further supported by the observations of Dedryver (1981, 1983). Comparing the three cereal-aphid species *M. dirhodum*, *S. avenae* and *R. padi*, that author found the latter species the least affected by fungi (mainly *P. neoaphidis*) under field conditions in France.

The economic injury level for *M. dirhodum* attacking wheat under South African growing conditions has not yet been quantified. However, data generated on spring-planted wheat in Idaho, USA, justified insecticide applications at *ca.* 4 aphids per tiller at the flowering stage, or *ca.* 10 aphids per tiller at the critical milk stage (Johnston & Bishop, 1987). At Bergville, the *M. dirhodum* population reached only 0.9 aphids per tiller at the flowering (1 October 1997) and 0.5 aphids per tiller at the milky ripe (8 October 1997) stages (Table 5.1), clearly well below injury levels. Feng *et al.* (1992b) found a negative correlation between aphid host density and infection level in > 75% of cases pertaining to *M. dirhodum*. This may explain the relatively high prevalence (20.6%) of infection recorded on this aphid (8 October) despite the low population density of aphids per tiller (< 1). As stated by Feng *et al.* (1992b), the negative correlation simply accentuates the role of Entomophthorales-induced epizootics in suppressing the increase of aphid populations rather than clues that host density is a useful predictor for fungal infection levels expressed as percentages.

5.4.2 Dryland wheat, winter rainfall (Langgewens)

With the addition of *Verticillium lecanii* (Zimmermann) Viégas, isolated from *D. noxia* during September 1996 (see Chapter 4), five species of aphid-pathogenic fungi were recorded from the winter-rainfall region during this study. Generally, the Hyphomycetes require high humidity conditions over several days for optimal sporulation and infection (Milner, 1997). The longterm daily mean relative humidity for September exceeds 68% in the Langgewens area, which, together with a median rainfall of 20 to 40 mm for that month (Schulze, 1997), most probably explains the occurrence of *B. bassiana* and *V. lecanii*. Surprisingly, neither of these hyphomycetous species was recorded from the warm irrigated conditions prevailing in the Bergville/Winterton area.

A total of five cereal-aphid species, including *D. noxia* (Table 5.1), were recorded from the Langgewens site on 5 September 1997. This represents the largest diversity of cereal aphids recorded from a single site during this study. However, *D. noxia* is not a major pest of wheat in the winter rainfall region of South Africa but rather is considered a secondary sporadic pest. In this region, *R. padi*, *S. avenae,* and *M. dirhodum* enjoy greater pest status, occurring annually from August to November (Prinsloo & Tolmay, 1999b). Of these species, *R. padi* is considered the most prevalent in the region (Walters, 1984; Walters *et al.*, 1988). This phenomenon was obvious during the Langgewens survey as *R. padi* comprised 89.2% of the total aphid population sampled (Table 5.2). Despite the high number of aphids the level of fungal infection in *R. padi* remained below 5%, as was the case under irrigated conditions in the Bergville/Winterton region. Furthermore, although the mean number of *R. padi* per sample unit was almost 9-fold higher than that of *D. noxia* (Table 5.1), fungal infection of *D. noxia* surpassed that of *R. padi* (14.3% versus 4.4%, respectively; Table 5.4). This observation suggests that *R. padi* may be less susceptible to *P. neoaphidis* than *D. noxia* as both aphid species occurred in the same microclimatic feeding niche, *i.e.,* inside rolled-up flag leaves (data not shown).

The economic importance of cereal aphids, especially *R. padi*, is underscored by the fact that during 1996 aphicide sales in this region were in excess of 3 million Rands (Mr. Johan Smit, Novon, personal comm.). In the winter-rainfall region damage to the wheat by *R. padi,* *S. avenae* and/or *M. dirhodum* can be expected when 70% or more plants become infested (Prinsloo & Tolmay, 1999b). From a control point of view, the data presented indicates that local species or strains of entomopathogenic fungi do not play a significant role in suppressing populations of *R. padi* in any of the three wheat-producing regions surveyed. It is believed that this phenomenon is likely to be related to some level of intrinsic low susceptibility of *R. padi* to these fungi. Epicuticular components are known to affect germination and virulence of several phytopathogenic fungi (Kumar & Sridhar, 1987; Conn & Tewari, 1989) as well as entomopathogenic fungi (Bidochka & Khachatourians, 1990, 1992; Hsiao & Khachatourians, 1997a; Inyang *et al.*, 1999). Ungerminated ballistospores, often observed on the cuticle of otherwise healthy *R. padi* feeding within tightly rolled-up flag leaves (favourable microclimate), may suggest the presence of inhibitory compounds on the cuticle surface of these aphids. *In vitro* studies with cuticular extracts (*e.g.* lipids) or with fractions of them are warranted. Moreover, cuticular melanization at the sites of fungal penetration (Brobyn & Wilding, 1977; Brey *et al.*, 1986; Hajek & St. Leger, 1994) is also considered as a highly possible aphid defense reaction (Humber, 1991), and should be investigated as a possible defense mechanism.

5.4.3 Dryland wheat, summer rainfall (Bethlehem)

During this study a total of four entomophthoralean species were recorded from cereal aphids under field conditions at Bethlehem, *i.e.*, *C. obscurus*, *C. thromboides*, *E. planchoniana* and *P. neoaphidis* (see also Chapter 4). Of these species the last was the most important pathogen infecting *D. noxia* during both 1996 (5, 14 and 20 November) and 1997 (3 November). From a total of 1 074 *D. noxia* cadavers examined during this period, > 99% were found infected by this pathogen (Tables 5.3 and 5.5). Shortly after *D. noxia* first appeared in the Free State province of South Africa, mycosed *D. noxia* were observed during the latter part of the wheat growth season, *i.e.*, under warm, moist summer conditions (probably November/December) (Walters *et al.*, 1980). The etiological agent was not identified by these authors but judging from the observations reported in this study, the responsible pathogen might have been *P. neoaphidis*.

In order to limit yield loss induced by *D. noxia* the wheat plant should be protected during the period between the emergence of the flag leaf (growth stage 37; see scale of Tottman, 1987) and the awn (growth stage 51) (Du Toit & Walters, 1984). For susceptible cultivars an insecticide application at growth stage 31 would generally be required for protection of the wheat plant during these critical stages (Du Toit & Walters, 1984; Prinsloo & Tolmay, 1999a). Therefore, control of *D. noxia* during early spring (September/October) is considered crucial (Prinsloo *et al.*, 1995) and for this reason late season epizootics (*i.e.*, during November; Table 5.4) are considered too late to effectively protect a susceptible wheat crop.

The effect of host-plant resistance on natural control of *D. noxia* by entomopathogenic fungi, especially by *P. neoaphidis* in the summer rainfall region, is still unknown. The genetic variations of host plants are known to affect the spatial dynamics of *D. noxia* (Schotzko & Smith, 1991; Schotzko & Quisenberry, 1998; Schotzko *et al.*, 1998) which, in turn, might also affect epizootic proliferation in space and time (Knudsen & Wang, 1998). Moreover, host plants are also known to alter susceptibility of insects to microbial pathogens (Benz, 1987). According to a study by Hsiao & Khachatourians (1997b) the fecundity of apterous adults of *R. padi,* artificially infected with the hyphomycete *V. lecanii,* was significantly associated with cereal host plant. Within the integrated-control programme, the development of a mycoinsecticide against *D. noxia* (see Chapter 6) will require tritrophic interaction studies to test the effect of antibiotic host-plant resistance on the susceptibility of this aphid.

5.5 Current review

The impact of fungal infection on an insect population is generally quantified by either collecting dead mycosed (overt) cadavers or by field-collecting live specimens for incubation under laboratory conditions (Eilenberg *et al.*, 1996). Observations during this study suggest that estimates of infection based on direct cadaver counts could be biased towards fungal species (*e.g.*, *P. neoaphidis*) effectively affixing their hosts to the plant substrate. Direct cadaver counts may lead to an underestimation of infection by species such as *Conidiobolus* spp., *B. bassiana*, and *V. lecanii* as these cadavers are weakly attached to the substrate only by their proboscises (see Chapter 4).

Diuraphis noxia and *M. dirhodum* seem highly susceptible to fungal infection with up to 50.0 and 77.1% infection recorded from these aphids, respectively. On the other hand, *R. padi* showed some level of low susceptibility to the indigenous aphid-pathogenic flora as the highest infection recorded from this aphid remained below 5%. Further studies are needed on what imparts resistance to these fungal pathogens by *R. padi*. In contrast, infection levels of

30-35% have been reported from *R. padi* in Denmark (Eilenberg *et al.*, 1996) and Hungary (Basky & Hopper, 2000). Surveys aimed at collecting *R. padi*-adapted species/strains of fungi for importation and subsequent release in the winter rainfall region of South Africa should be pursued.

Under dryland conditions in the summer-rainfall region the entomophthoralean, *P. neoaphidis*, was the most important pathogen species attacking *D. noxia.* Recent attempts at formulating this pathogen for use as a mycoinsecticide against aphids yielded good results (Shah *et al.*, 1999) but generally the Hyphomycetes are considered better candidates in this regard (Milner, 1997). Although the synergistic use of mycoinsecticides and host-plant resistance against *D. noxia* is still in its infancy (Knudsen & Wang, 1998; Vandenberg *et al.*, 2001) the two Hyphomycetes, *B. bassiana* and *P. farinosus* isolated from *D. noxia* during this study, should be further investigated for their potential use in the integrated-control programme (see Chapter 6).

CHAPTER 6

EFFICACY OF *Beauveria bassiana* (HYPHOMYCETES) FOR CONTROL OF RUSSIAN WHEAT APHID (HOMOPTERA: APHIDIDAE) ON RESISTANT WHEAT UNDER FIELD CONDITIONS

6.1 Introduction

Host-plant resistance against the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), was reported for the first time in South Africa by Du Toit (1987). Several resistant wheat cultivars have since been bred for commercial use against this aphid, worldwide (Souza, 1998; Bonjean & Angus, 2001), with 15 such cultivars released in South Africa since 1992 (Tolmay, 2001). In South Africa, these resistant cultivars form the basis of an integrated-control programme being developed by the Small Grain Institute (SGI) of the South African Agricultural Research Council (ARC). By lowering the frequency of chemical control applications (Marasas, 1999), the widespread deployment of host-plant resistance is expected to promote the additive effects of both indigenous and introduced natural antagonists of this aphid (Marasas *et al.*, 1997).

A preliminary field trial conducted in 1997 with the entomopathogenic fungus *Beauveria bassiana* (Balsamo) Vuillemin against *D. noxia* on susceptible wheat (dryland conditions), showed that a significant reduction in the number of aphids could be attained after two consecutive (seven-day interval) applications of the fungus (Hatting & Poprawski, 1998). Recently, Vandenberg *et al.* (2001) also reported a significant reduction in both the percentage *D. noxia*-infested tillers and number of aphids per tiller 14 days after application of *B. bassiana* on susceptible wheat under irrigated conditions.

Knudsen *et al.* (1994) studied the tritrophic interaction between *D. noxia*, *B. bassiana*, and a non-preferred host plant (oats, *Avena sativa* L.) versus a preferred host plant (wheat, *Triticum aestivum* L.). This interaction was found to be significant for total nymph production, in that the proportionate reduction of the aphid population by the fungus was greater on the non-preferred host. These results support a hypothesis that the efficacy of fungal entomopathogens might be positively affected by the use of resistant wheat varieties. In this regard, reduced leaf rolling (Tolmay *et al.*, 1999) may result in greater exposure of aphids to spray applications; greater tolerance to aphids could provide slow acting fungi with more time to infect and kill the host before economical injury is caused to the crop, while antibiotic resistance will lead to slower development (*i.e.*, extended periods between ecdysis) of the insect pest (Smith, 1989), thereby providing fungi with more time to penetrate the cuticle. Only one other published report of *B. bassiana* efficacy against *D. noxia* on resistant wheat exists. Vandenberg *et al.* (2001) reported that *B. bassiana* strain GHA was ineffective against *D. noxia* infesting the resistant spring wheat cultivar 'IDO-488'.

Field trials were conducted under dryland conditions during the 1998 and 1999 seasons to assess the aphid-control efficacy of spray applications of *B. bassiana* in combination with host-plant resistance. In addition to aphid population densities and percentage tiller infestation, grain yield was also measured for comparison with existing chemical-trial data being gathered by ARC-SGI.

6.2 Material and methods

6.2.1 Trial design and layout

Both trials were conducted at the Small Grain Institute (SGI) of the Agricultural Research Council (ARC) of South Africa, Bethlehem, Free State Province. A plot of $800m^2$ (20m x 40m) was planted with the antibiotic cultivars 'Limpopo' and 'Elands' (Basky, 2002) at a seeding density of 25 kg/ha during 1998 and 1999, respectively. Natural aphid infestations occurred in both years. The two trials were designed as completely randomised designs (Cochran & Cox, 1957) with 8 (one treatment and an untreated control, each replicated four times) and 12 (two treatments and an untreated control, each replicated four times) sub-plots, respectively, during 1998 and 1999. Each sub-plot measured $10m^2$ (2.5m x 4m).

6.2.2 Fungus and delivery rate

A commercial preparation of *B. bassiana* strain GHA formulated as a paraffinic oil-based emulsifiable suspension (Mycotrol® ES produced by Mycotech Corp., Butte, Montana, USA) containing 2.1×10^{13} conidia per litre was applied at a rate of 2.4 litres (5×10^{13} conidia) per ha. Percentage germination, verified prior to application during both years, exceeded 97%. Mycotrol® ES was applied by means of a CO_2-pressurized back-pack sprayer fitted with 5 single flat-fan nozzles (nr. 8001. TeeJet Spray Systems Company, Wheaton, Illinois, USA) spaced *ca.* 50cm apart on a single boom at a walking speed delivering 350 l/ha at 200kPa. Nozzles were directed directly downward, delivering the spray suspension from a height of *ca.* 70cm above the soil surface. The organosilicone nonionic surfactant, Break-Thru® (Polyether-polymethylsiloxane-copolymer) (Goldschmidt Chemical Corporation, Hopewell, VA, USA), was added to the spray suspension at a rate of 0.1%, *i.e.*, the registered full-rate for use with certain herbicides under field conditions in South Africa (Syngenta, Reg. no. L5895). Control plots were left untreated, although a carrier assay was performed under greenhouse conditions to investigate the potential impact of the carrier agents on *D. noxia* (see 6.2.5).

6.2.3 Spray applications, aphid counts and data analyses

During 1998, aphid counts were conducted on five dates, *i.e.*, 98/10/01 (pre-spray), 98/10/14, 98/10/23, 98/10/30 and 98/11/06. Mycotrol® ES was applied on growth stage 31 (*i.e.*, first node detectable; 98/10/02) and again on growth stage 39 (*i.e.*, flag leaf ligule just visible; 98/10/15) (growth stages according to Tottman, 1987). Both spray applications were administered just after sunset. During the 1999 trial, five counts were again executed, *i.e.*, on 99/10/04 (pre-spray), 99/10/18, 99/10/29, 99/11/08, and 99/11/17. Treatment 1 received a Mycotrol® ES application on growth stage 31 (after sunset, 99/10/05) and again on growth stage 39 (early morning, 99/10/20) while Treatment 2 received only one spray application on growth stage 39 (early morning, 99/10/20).

During each count, two plants were selected (nondestructive) in each of five rows totalling 10 plants per plot. Selection of plants was performed systematically within each row: on the initial sample date, plants number 5 and 25 were selected in each row; for each subsequent sample, the position was shifted by 5 plants so that no plant was sampled more than once.

During each count the following parameters were quantified: 1) number of tillers per plant, 2) number of *D. noxia*-infested tillers per plant, and 3) total number of adult (winged and wingless) and nymphal aphids per tiller. The total number of aphids per 10 plants for each plot was used in the analysis. Data were analysed using the statistical program GenStat for Windows (2000). One-way analysis of variance (ANOVA) was used to test for differences between the control and treated plots. However, due to pre-spray differences detected in the total number of *D. noxia* in treatment versus control plots during 1998 an additional analytical method was used to assess potential treatment effects during that year. Usually, the preferred method of analysis of treatment effects under these circumstances would be analysis of covariance (ANCOVA). ANCOVA adjusts the post-spray counts for the linear relationship with the pre-spray counts (X). An important assumption for the use of ANCOVA, however, is that the regression of aphid density on sample date for each group (treatment versus control) must have a common slope (Snedecor & Cochran, 1980), and in this study, this assumption did not hold true. An alternative to the use of ANCOVA is the use of Y – X, the change in the count, as the measure of treatment effect (Snedecor & Cochran, 1980). Unfortunately, analysis of Y – X may be inferior to the simple analysis (comparison) of Ys, if the correlation between X and Y is low (Snedecor & Cochran, 1980), and this was also the case in this study. After considering these various difficulties, it was decided to assess potential treatment effects by application of more than one analytical method. Thus, analysis of variance was used to compare changes in pre- versus post-treatment means (Y – X where $Y_i = 1^{st}$ count post application; $Y_{ii} = 2^{nd}$; etc.) and also to directly compare unadjusted post-treatment means. Moreover, in view of these pre-treatment differences, corrected percentage efficacies were calculated according to the following modification of Abbott's formula as described by Henderson & Tilton (1955):

% Efficacy = [1 – (Ta/Ca x Cb/Tb)] x 100

Where: Tb = infestation in treated plot prior to application
Ta = infestation in treated plot after application
Cb = infestation in control plot prior to application
Ca = infestation in control plot after application

Multiple treatment means were separated using the Tukey Honestly Significant Difference (HSD) test at the 5% level of significance (Snedecor & Cochran, 1980). For percentage infested tillers the arcsine transformation was used, and for number of aphids, the log transformation was used to stabilise treatment variances. All plots were harvested for yield comparison (g/plot), and data were analysed using the statistical program GenStat for Windows (2000).

6.2.4 Weather data

Daily weather data were recorded at the Bethlehem Loch Lomond weather station situated at ARC-SGI. During both 1998 and 1999, the wheat fields used in the trials were within 100m of this station. All weather data were supplied by the Agromet Section of the ARC-Institute for Soil, Climate and Water, Pretoria, South Africa.

6.2.5 Carrier assay

Twenty ml of Mycotrol® ES was poured into a 100ml glass beaker and heated to 95°C for *ca.* 5 seconds over a gas flame. The product was cooled to 20°C before being re-heated. The procedure was repeated ten times. Conidial mortality was assessed by inoculating a plate of Sabouraud dextrose agar (SDA) + 1% yeast extract with 1µl of (heat-treated) Mycotrol® ES plus 2.5ml sterile water. The plate was incubated at 25°C for 24 hours and the percentage germination verified under a light microscope using 400X magnification. This procedure resulted in 100% conidial mortality.

Diuraphis noxia was collected from ARC-SGI stock colonies maintained on the susceptible wheat cultivar 'Betta' at 23°C ± 1°C, 70 ± 5% RH, under natural light conditions. Fifteen batches of 50 aphids each comprising instar 3 – 5 (apterous, mixed) were used to infest wheat plants (growth stage 23; cultivar 'Betta') growing in 350ml, 9cm-diameter pots (3 plants per pot). Each batch of aphids was scattered onto the soil surface of a given pot and allowed 12 hours to ascend and settle on the seedlings. Five pots (replications) were allocated to each treatment and control. Each pot with resident aphids was sprayed individually using a precision spray tower constructed after Burgerjon (1956) (see Chapter 7, section 7.2.2.3). Treatment 1 received a water volume and rate of heat-treated Mycotrol® ES plus organosilicone surfactant similar to that used during the field trials (see 6.2.2). Treatment 2 received an application of only the organosilicone surfactant, whereas controls received a water-only application equal to 350 l/ha. For calibration purposes, the delivery rate of the spray tower was calculated by weighing water deposits (see Liu & Stansly, 1995).

Immediately after spraying, treated pots were placed under open-air conditions in the greenhouse at a day:night temperature cycle of 21°C ± 2°C : 12°C ± 2°C, 70% ± 5% RH, under natural light. Pots were examined after 72 hours and the number of live (excluding instar 1 and 2), dead and missing aphids recorded.

The number of missing aphids (due to non-caged, open-air conditions) for each treatment and control was analysed by one-way analysis of variance (ANOVA) using the statistical program GenStat for Windows (2000). Treatment means were separated using the Tukey Honestly Significant Difference (HSD) test at the 5% level of significance (Snedecor & Cochran, 1980). Percentages of mortality were corrected according to Abbott's formula (Abbott, 1925) using mortality in the untreated control as the correction factor. Corrected percentage mortality was normalized through arcsine transformation and the angular values of mortality analyzed by one-way analysis of variance using the statistical program GenStat for Windows (2000). Treatment means were separated using the Tukey Honestly Significant Difference test at the 5% level of significance (Snedecor & Cochran, 1980).

6.3 Results

6.3.1 1998 Trial

Compared with the pre-spray counts for 1999 (see 6.3.2) the natural aphid infestation within plots varied considerably. Most importantly, the pre-spray count (98/10/01) indicated a significant difference in the total number of *D. noxia* in treatment versus control plots ($P = 0.011$; Table 6.1).

The comparative change ($Y_i - X$) in the number of *D. noxia*, recorded 12 days post application (*i.e.*, on 98/10/14), was not significantly different in treated versus control plots ($F = 4.64$ on 1, 6 df; $P = 0.075$). However, some level of control was evident, as the aphid population had increased *ca.* 3.5-fold on controls compared to a 1.5-fold increase in treated plots. This increase, in favour of controls, is underscored by the fact that no significant difference ($P = 0.424$; Table 6.1) was detected in the number of aphids in treated vs control plots on 98/10/14, suggesting a significant shift in aphid densities compared with 98/10/01.

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This effect was further amplified following the second application on 98/10/15. During the eight days following this treatment, the aphid population in the treated plots declined by 38%, while the population in the control plots increased by 6% (Fig. 6.1). These fluctuations ($Y_{ii} - X$) differed significantly ($F = 49.29$ on 1, 6 df; $P < 0.001$), as did the actual number of aphids recorded per plot on 98/10/23 ($P = 0.005$; Table 6.1).

Table 6.1. Mean number of *D. noxia* recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 litres (5 x 10^{13} *B. bassiana* conidia) per hectare + 0.1% Break-Thru®. 1998

DATE:	98/10/01[a]	98/10/14	98/10/23	98/10/30	98/11/06
Control	32.0 ± 4.1b[b]	113.8 ± 61.3a[b]	120.8 ± 24.5b[b]	49.5 ± 21.8a[b]	16.8 ± 3.4a[b]
Treatment	53.8 ± 12.6a	80.8 ± 18.6a	50.5 ± 20.2a	28.5 ±11.4a	8.5 ± 7.1a
Efficacy[c]	---	29.0	58.2	42.4	49.4
Corrected Efficacy[d]	---	55.5 ± 16.7	74.9 ± 7.9	64.0 ± 17.6	66.5 ± 30.0
P	0.011	0.424	0.005	0.104	0.059
F (1, 6 df)	13.26	0.74	19.34	3.67	5.39

[a] Pre-spray count. Spray applications administered on 98/10/02 and 98/10/15.

[b] Log 10 transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within a column are not significantly different by ANOVA at the 5% level.

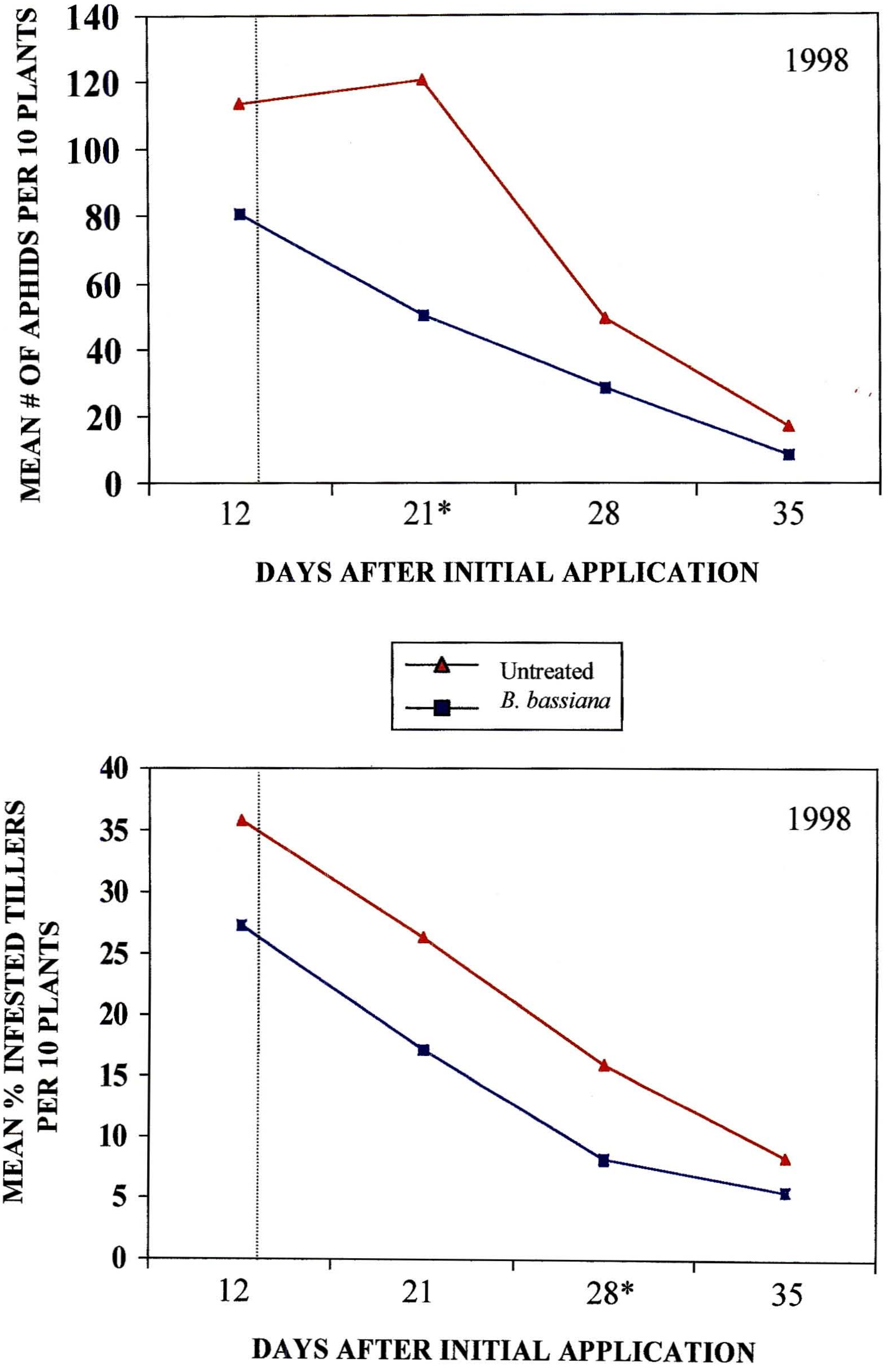
[c] Percent reduction in aphid population relative to the control.

[d] Corrected efficacy ± standard error calculated according to the formula of Henderson & Tilton (1955).

Expressed as a percentage of the control, efficacy of the treatments averaged 44.8% over the duration of the trial, but this value is likely to be a conservative estimate because of the higher pre-spray aphid densities recorded in the treatments (Table 6.1). After applying the Henderson-Tilton correction, efficacy averaged *ca.* 65%. A significant treatment effect was also observed by comparing the cumulative number of aphids recorded over the last three sample dates, *i.e.*, 87.5 versus 187.0 aphids per 10 plants for treatment and control plots, respectively (F = 15.41 on 1, 6 df; P = 0.008).

In contrast to the findings with population densities, no significant difference was detected between the pre-spray infestation levels in the control versus treatment plots (P = 0.339) (Table 6.2). No significant differences in percentage infested tillers were detected until day 28, when *ca.* 50% fewer tillers were found infested compared with controls (P < 0.001; Table

Figure 6.1. Mean number of aphids and percentage infested tillers recorded per 10 plants for trial in 1998



ASTERISKS INDICATE SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS AND CONTROLS AT THE 5% LEVEL OF SIGNIFICANCE

DOTTED LINE INDICATES SECOND MYCOTROL® APPLICATION

6.2 & Fig. 6.1). A 35% lower rate of infestation was recorded in the treatment plots relative to the controls on 98/10/23. This difference was not significant at the 5% level but was significant at the 10% level (P = 0.069), and a highly significant 48% lower rate of infestation was observed in the treatment plots in the subsequent sample (98/10/30) (Table 6.2). By day 35 post application, the wheat was less palatable (*ca.* growth stage 70), resulting in very low aphid densities (< 3 aphids per plant). Subsequently, sampling was terminated following the fifth count on 98/11/06.

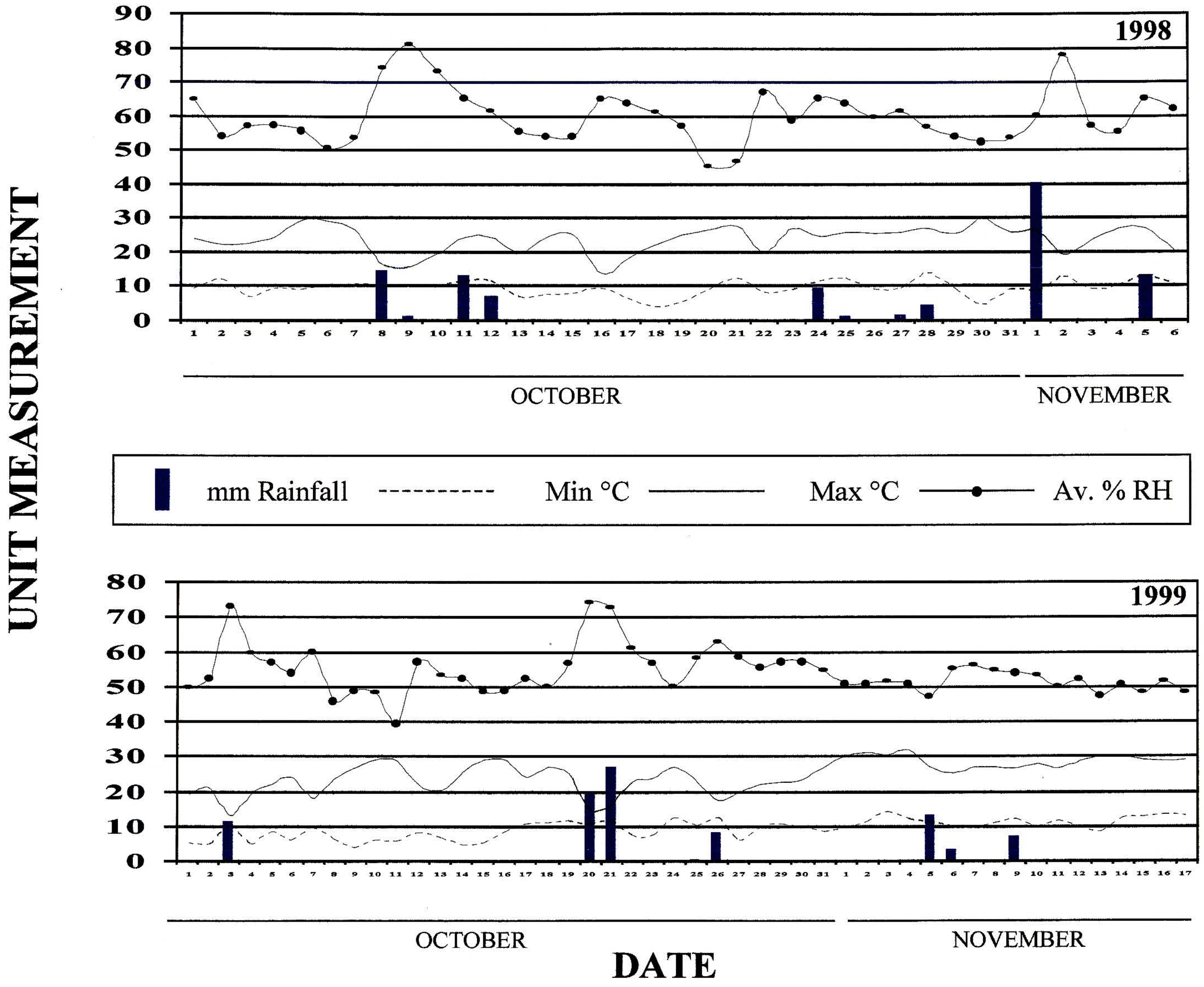
Table 6.2. Percentage tiller infestation recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 litres (5 x 10^{13} *B. bassiana* conidia) per hectare + 0.1% Break-Thru®. 1998

DATE:	98/10/01[a]	98/10/14	98/10/23	98/10/30	98/11/06
Control	21.4 ± 3.2a[b]	35.8 ± 7.4a[b]	26.4 ± 6.2a[b]	16.0 ± 2.4b[b]	8.4 ± 3.0a[b]
Treatment	18.9 ± 3.8a	27.3 ± 6.7a	17.1 ± 6.0a	8.3 ± 0.9a	5.5 ± 2.0a
Efficacy[c]	---	23.7	35.2	48.1	34.5
P	0.339	0.135	0.069	<0.001	0.135
F (1, 6 df)	1.08	2.97	4.90	42.99	2.97

[a] Pre-spray count. Spray applications administered on 98/10/02 and 98/10/15.
[b] Arcsine transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.
[c] Percent reduction in the rate of tiller infestation relative to the control.

Weather conditions remained stable throughout the duration of the trial with average daily minimum and maximum temperatures of 10.2°C (range = 4.3 – 14.5°C) and 23.9°C (range = 13.9 – 30.3°C), respectively, measured for the (combined) months of October and November. A single heavy rainfall of 40.2mm was measured on 98/11/01 (Fig. 6.2), but this occurred when aphid populations were already declining as a result of wheat ripening. Grain yields averaged 2 170 (SE = 215.9) and 1 976 (SE = 177.4) g/plot for treatments and controls, respectively, and did not differ significantly (F = 1.919 on 1, 6 df; P = 0.2153).

Figure 6.2. Daily weather data recorded for trials in 1998 and 1999



6.3.2 1999 Trial

The number of aphids recorded on 99/10/04 (pre-spray), averaged 52.3 (SE = 31.3) with no differences among treatments and control (P = 0.977; Table 6.3). In contrast to the trend observed in 1998, the first Mycotrol® application on 99/10/05 (*i.e.*, Treatment 1) resulted in no significant shift among aphid populations when compared with the (as then) unsprayed second treatment and/or control on 99/10/18 (P = 0.809; Table 6.3).

Table 6.3. Mean number of *D. noxia* recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 litres (5 x 10^{13} *B. bassiana* conidia) per hectare + 0.1% Break-Thru®. 1999

DATE:	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	52.5 ± 31.9a[b]	63.0 ± 47.3a[b]	16.5 ± 1.0a[b]	7.0 ± 3.6a[b]	6.5 ± 3.1a[b]
Two applications	51.3 ± 41.3a	55.3 ± 45.0a	9.3 ± 6.6ab	5.8 ± 5.6a	6.5 ± 2.5a
One application	53.3a ± 29.6a	65.5a ± 32.7a	3.3b ± 2.1b	3.5a ± 4.0a	3.8a ± 1.0a
P	0.977	0.809	0.021	0.295	0.189
F (2, 9 df)	0.02	0.22	6.11	1.40	2.01
$HSD_{(5\%)}$	---	---	0.485	---	---

Treatments included 1) untreated control, 2) two fungus sprays applied 15 days apart on 99/10/05 and 99/10/20, and 3) one fungus spray applied 99/10/20.

[a] Pre-spray count.

[b] Log 10 transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within the same column are not significantly different by Tukey's HSD Test at the 5% level.

Compared with the control, the only significant difference in both the number of aphids (P = 0.021; Table 6.3) and percentage infested tillers (P = 0.049; Table 6.4) was observed with Treatment 2 nine days after the single application on 99/10/29. However, in view of the rainfall events experienced during 1999 (resulting in very low aphid densities after 99/10/18) and the fact that no differences were detected between the two treatments (spray application on GS 31 & 39 versus GS 39 only) on any date post application (Table 6.3 and 6.4), data for Treatment 1 and 2 were pooled (analysed as a single treatment) for each date.

Table 6.4. Percentage tiller infestation recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 litres (5×10^{13} *B. bassiana* conidia) per hectare + 0.1% Break-Thru®. 1999

DATE:	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	27.6 ± 11.4a[b]	27.3a ± 11.8a[b]	11.9a ± 6.8a[b]	5.5a ± 1.1a[b]	6.6a ± 1.5a[b]
Two applications	25.3 ± 16.5a	22.7 ± 15.0a	8.3 ± 2.9ab	4.1 ± 2.5a	5.8 ± 4.0a
One application	26.8 ± 14.0a	31.7 ± 13.3a	3.6 ± 2.4b	3.0 ± 3.4a	3.6 ± 0.7a
P	0.957	0.631	0.049	0.253	0.167
F(1, 10 df)	0.04	0.48	4.30	1.61	2.20
HSD(5%)	---	---	8.77	---	---

Treatments included 1) untreated control, 2) two fungus sprays applied 15 days apart on 99/10/05 and 99/10/20, and 3) one fungus spray applied 99/10/20.

[a] Pre-spray count.

[b] Arcsine transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within the same column are not significantly different by Tukey's HSD Test at the 5% level.

The pooled data indicated a significant difference in the number of aphids recorded only on 99/10/29 (P = 0.040; Table 6.5); numbers in the treated plots were 63% lower than in the untreated plots. This level of control (following a Mycotrol® application during the flag-leaf stage) was attained despite a dramatic reduction (> 80%) in aphid densities in all plots recorded between 99/10/18 and 99/10/29 (Fig. 6.3). The only obvious explanation for this phenomenon is the adverse weather conditions experienced on 99/10/20 and 99/10/21 (Fig. 6.2). During this period, a total of 47mm of rain was recorded, coinciding with an average temperature of only 13.3°C.

Table 6.5. Mean number of *D. noxia* recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 litres (5 x 10^{13} *B. bassiana* conidia) per hectare + 0.1% Break-Thru®. Pooled data (*i.e.*, Treatment 1 and 2) for 1999

DATE:	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	52.5 ± 31.9a[b]	63.0 ± 47.3a[b]	16.5 ± 1.0b[b]	7.0 ± 3.6a[b]	6.5 ± 3.1a[b]
Treatments	52.3 ± 33.3a	60.4 ± 36.8a	6.3 ± 5.5a	4.6 ± 4.7a	5.1 ± 2.3a
Efficacy[c]	---	4.2	62.9	32.1	21.2
P	0.889	0.969	0.040	0.245	0.441
F (1, 10 df)	0.02	0.002	5.60	1.52	0.64

[a] Pre-spray count. Spray applications administered on 99/10/05 and 99/10/20.
[b] Log 10 transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.
[c] Percent reduction in aphid population relative to the control.

No significant difference in the percentage infested tillers was detected for the pooled data on any of the survey dates during 1999 (Table 6.6 & Fig. 6. 3). Grain yields averaged 1 846 (SE = 150.0), 1 965 (SE = 78.2), and 1 910 (SE = 135.7) g/plot for Treatments 1, 2 and controls, respectively, and did not differ significantly (F = 0.913 on 2,9 df; P = 0.4353).

Table 6.6. Percentage tiller infestation recorded per 10 plants pre- and post-application of Mycotrol® ES at a rate of 2.4 litres (5 x 10^{13} *B. bassiana* conidia) per hectare + 0.1% Break-Thru®. Pooled data (*i.e.*, Treatment 1 and 2) for 1999

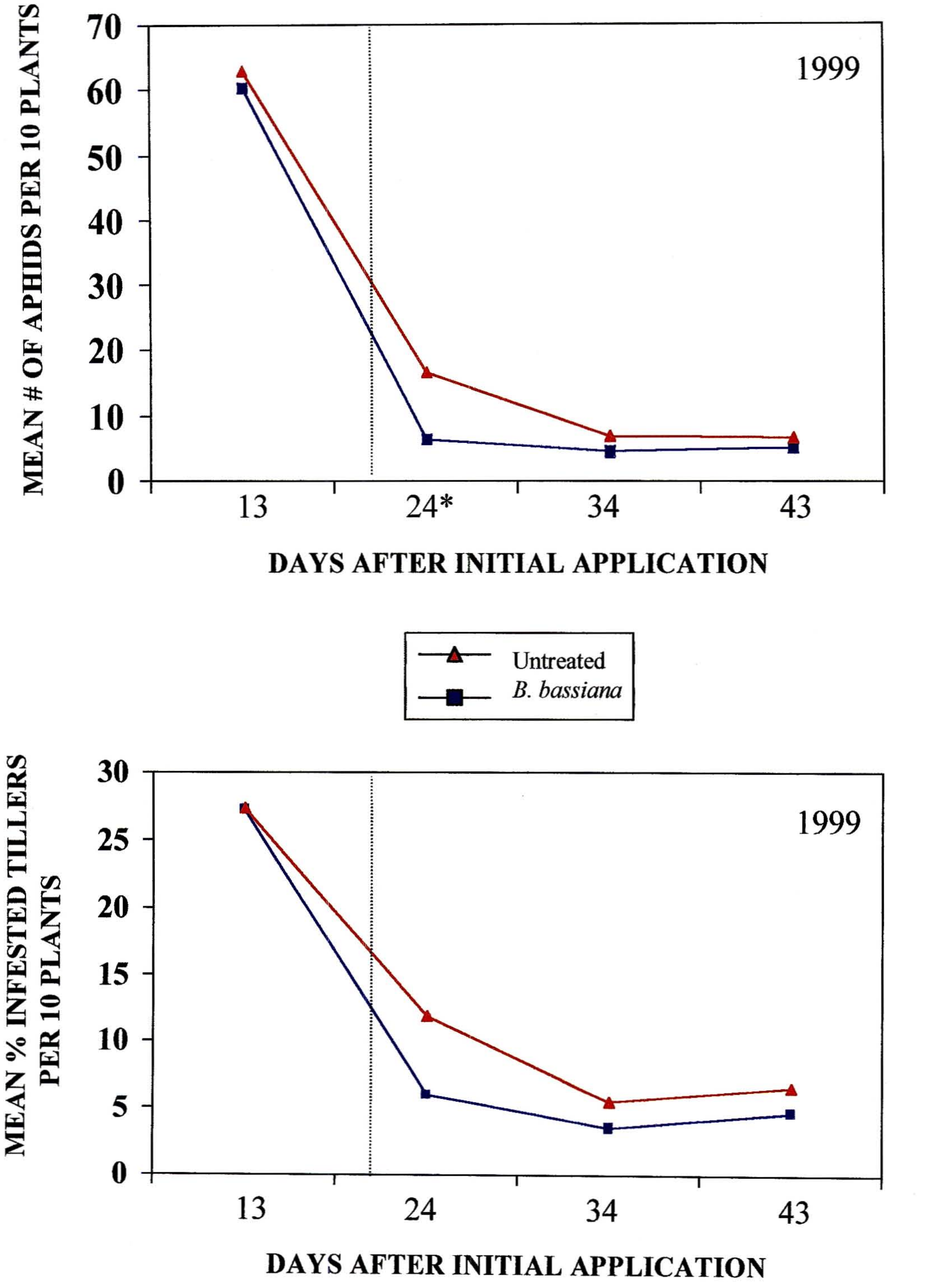
DATE:	99/10/04[a]	99/10/18	99/10/29	99/11/08	99/11/17
Control	27.6 ± 11.4a[b]	27.3 ± 11.8a[b]	11.9 ± 6.8a[b]	5.5 ± 1.1a[b]	6.6 ± 1.5a[b]
Treatments	26.0 ± 14.2a	27.2 ± 14.0a	6.0 ± 3.5a	3.5 ± 2.9a	4.7 ± 2.7a
Efficacy[c]	---	0.4	49.6	36.4	28.8
P	0.800	0.963	0.080	0.211	0.159
F (1, 10 df)	0.07	0.002	4.30	1.78	2.31

[a] Pre-spray count. Spray applications administered on 99/10/05 and 99/10/20.

[b] Arcsine transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.

[c] Percent reduction in tiller infestation relative to the control.

Figure 6.3. Mean number of aphids and percentage infested tillers recorded per 10 plants for trial in 1999



ASTERISKS INDICATE SIGNIFICANT DIFFERENCE BETWEEN TREATMENTS AND CONTROLS AT THE 5% LEVEL OF SIGNIFICANCE

DOTTED LINE INDICATES SECOND MYCOTROL ® APPLICATION

6.3.3 Carrier assay

Aphid counts conducted 72 hours after treatment revealed, on average (± SE) 5.6 ± 6.9, 7.6 ± 3.6, and 6.6 ± 6.2 aphids missing from control, Treatment 1 and Treatment 2, respectively (Table 6.7). As these values did not differ significantly (F = 0.15 with 2, 12 df; P = 0.861), the percentage mortality was calculated as [number of dead aphids] / [total number of aphids – missing aphids] x 100, and then corrected according to Abbott's formula (Table 6.7). Abbott-corrected arcsine transformed mortalities averaged 23.0 and 11.8% for Treatment 1 and 2, respectively. This difference was highly significant (P = 0.005; Table 6.7).

Table 6.7. Carrier-induced mortality of *D. noxia* 72 hours post treatment

	MEAN[a] NUMBER OF			MEAN MORTALITY	
Treatment	Alive	Dead	Missing	%[a]	%[b]
Control	43.4 ± 6.1	1.0 ± 1.0	5.6 ± 6.9	2.04 ± 2.0	---
Surfactant	39.6 ± 4.0	2.8 ± 1.1	7.6 ± 3.6	6.7 ± 2.7	4.5 ± 2.8a
Surfactant + Oil	36.0 ± 7.3	7.4 ± 1.9	6.6 ± 6.2	17.6 ± 6.5	15.7 ± 6.6b
P					0.005
F(1, 8 df)					14.95

[a] Mean ± standard error of 5 replicate pots. 50 Aphids per pot.

[b] Abbott-corrected percentage ± standard error. Arcsine transformation applied prior to ANOVA. Means ± standard errors followed by the same letter within the same column are not significantly different by ANOVA at the 5% level.

6.4 Discussion

By applying Mycotrol® ES (+ 0.03% Silwet surfactant) at weekly intervals against *D. noxia* feeding on a susceptible cultivar ('Tugela'), Hatting & Poprawski (1998) demonstrated a stabilizing effect on *D. noxia* population growth following 2 and 3 consecutive applications. The greatest reduction in the number of *D. noxia* (59% compared with the control) was attained after the third application, *i.e.*, during the early flag-leaf stage (GS 38). Notably, an application after colonization and subsequent rolling of the flag-leaves (*ca.* GS 45; boots swollen) did not prevent aphids from increasing significantly, from 21 to 45 aphids per tiller. The most serious damage inflicted by *D. noxia* normally occurs during the period between the emergence of the flag leaf (GS 37) and the awn (GS 51) (Du Toit & Walters, 1984, Fouché *et al.*, 1984). For this reason, chemical control of *D. noxia* on susceptible wheat, is recommended at growth stage 31 when 7 - 14% plants are infested (Du Toit, 1986; Prinsloo & Tolmay, 1999a), thereby protecting the upper two leaves from aphid infestation (Kriel *et al.*, 1984). Although the first mycoinsecticide application by Hatting & Poprawski (1998) was correctly timed in terms of this threshold (applied at GS 31), protection of the upper leaves was not satisfactory even though subsequent applications were made at weekly intervals. Hatting & Poprawski (1998) concluded that the poor level of control throughout the critical flag-leaf stage was mainly attributed to the rapid increase in aphid numbers after colonization of the flag leaf (Kriel *et al.*, 1986) and aphid habitation of a cryptic site (within the rolled flag leaves) which possibly shielded the aphids from subsequent spray applications.

It was hypothesized in the present study that the use of a mycoinsecticide in combination with host-plant resistance would largely address the abovementioned impediments. During both 1998 and 1999, an average of 60.6 ± 3.3% aphid control was observed following a fungus application during the early flag-leaf stage of the resistant wheat cultivars employed in this study. Surprisingly, however, Vandenberg *et al.* (2001) observed no significant efficacy of *B. bassiana* strain GHA applied against *D. noxia* on the resistant wheat cultivar 'IDO-488'. The reasons for this substantial difference are unknown. Vandenberg *et al.* (2001) suggested that the reduced leaf-rolling response on the resistant variety might have resulted in a microclimate less favourable to fungal infection of *D. noxia*. On the other hand, observations of similar levels of control on both susceptible (Hatting & Poprawski, 1998; Vandenberg *et al.*, 2001) and resistant varieties (present study) suggest either that plant architecture is not an important factor determining fungal efficacy, or that greater exposure of aphids to spray applications and the loss of favourable (shaded, humid) conditions due to reduced leaf roll have offsetting effects. Perhaps more importantly, the actual timing of fungus application needs to be considered. As pointed out above, a late fungus application after colonization and subsequent rolling of the flag leaves (GS 45) did not prevent aphids from increasing significantly on the susceptible cultivar (Hatting & Poprawski, 1998). Unfortunately, no indication of the actual timing of application in terms of wheat growth stage was given by Vandenberg *et al.* (2001). The most effective control observed by Hatting & Poprawski (1998) followed applications prior to and during the early flag-leaf stage when little leaf roll was evident and aphids were actively migrating onto the flags.

These observations lead to an alternative hypothesis that might account for greater efficacy of fungal sprays applied prior to colonization of the flag leaf. Aphids migrating to the flag leaf may acquire lethal doses of conidia from the plant surface (Hall, 1979; Roditakis *et al.* 2000). The greater change in aphid numbers that occurred following the initial spray in 1998 (significant at the 8% level; P = 0.075; corrected efficacy of 56%; Table 6.1) than in 1999 (P = 0.97; efficacy of 4%; Table 6.5) may also support this hypothesis, as the cultivar used in 1998 ('Limpopo') supported a higher level of aphid activity than did the cultivar used in 1999 ('Elands'). On cultivar 'Limpopo' the average percentage tiller infestation in untreated plots increased from 21.4% (pre-spray) to 35.8% within 12 days (Table 6.2). In contrast, tiller infestation during a comparable period of time remained at 27% on cultivar 'Elands' during 1999 (Table 6.6). During these two time periods, ambient temperature conditions were highly comparable, with daily average minimum and maximum temperatures recorded for 1998 and 1999 ranging from 9.7°C – 23°C and 7.1°C – 24.5°C, respectively. Although four rainfall events were recorded during 1998, these were of low intensity, averaging only 8.9 ± 6.1mm per event. No rainfall was recorded during this time period for 1999.

Similarly, the acquisition of conidia from plant surfaces during migration from infested to uninfested tillers may explain the higher level of control following the early spray application during 1998. This higher level of migration is, in turn, attributed to the lower level of antibiotic resistance in cultivar 'Limpopo' compared with cultivar 'Elands' (Basky, 2002). Aphid numbers in untreated plots increased 3.5-fold in 12 days on cultivar 'Limpopo' compared with a 1.2-fold increase over a 13 day period on cultivar 'Elands' (Table 6.1 & 6.5). In view of the many different resistant cultivars commercially available in South Africa (Tolmay, 2002), studies aimed at better understanding the tritrophic interactions between host plant, pest and pathogen are warranted. In this regard, a novel bioassay methodology was developed at ARC-SGI and is reported in detail in Chapters 7 and 8 of this thesis.

Following field observations during the 1997 trial (see Hatting & Poprawski, 1998), it was also decided to use a higher rate of an organosilicone-type surfactant in order to optimize wetting of the target. Lo & Hopkinson (1995) studied the surface tension, contact angle and spread area of six concentrations ranging from 0.001 to 1.0% of three surfactants (including the organosilicone-type nonionic surfactant Silwet L-77) on three low-energy solid surfaces. These authors concluded that the *minimum* concentration needed for spontaneous spreading ranged from 0.05 to 1% for the organosilicone-type surfactant. However, due to economic implications, a rate exceeding the registered full-rate of 0.1% was not feasible under field conditions in South Africa. On the other hand, the insecticidal activity of organosilicone-type surfactants (Imai *et al.*, 1994, 1995; Purcell & Schroeder, 1996; Poprawski *et al.*, 1999) and oils (Davidson *et al.*, 1991 and references within) is well documented, especially against soft-bodied insects such as aphids. When considering the efficacy of the fungus *per se*, the 15.7% Abbott-corrected mortality observed following application of these carriers in the laboratory must, therefore, be taken into consideration and additional study under field conditions is warranted. Although field trials conducted by Vandenberg *et al.* (2001) included both water-sprayed and untreated controls, no effects of the oil or organosilicone formulants were measured. Future trials should include a spray-carrier control to quantify the impact of such carriers under field conditions (Poprawski *et al.*, 1999). Observations made under both greenhouse and field conditions with this study, revealed no phytotoxicity on wheat at the 0.1% rate used.

Only one other report on field trials with a hyphomycete against Russian wheat aphid has been published. Knudsen & Wang (1998) used pellet-formulated *B. bassiana* (Knudsen *et al.*, 1990) in four trials over two seasons. Although >95% of pellets were observed to have sporulated on the soil surface during one trial, a maximum high of only 18% mortality was recorded. The restricted movement of infective propagules into the crop canopy was considered the main reason for this low level of control obtained. The abovementioned trial by Vandenberg *et al.* (2001) included two foliar-applied formulations of *B. bassiana* and *Paecilomyces fumosoroseus* (Wize) Brown & Smith under irrigated conditions against *D. noxia* feeding on susceptible wheat. They observed reduction in aphid population densities within 14 days post application in most experiments with spring wheat for three consecutive years (1995 – 1997). The highest level of control recorded by these authors during their 1997 trial (*i.e.*, spring wheat; large-plot experiment) was *ca.* 60%, comparable with the 58.2% (uncorrected) and 62.9% reported here for 1998 and 1999, respectively. Notably, this level of control was also recorded about two weeks after application of the fungus, similar to the observations made during this study. These findings accentuate the slow acting nature of the fungus under field conditions.

During 1995, ARC-SGI initiated a long-term field evaluation programme to measure the yield and quality response of *D. noxia*-resistant cultivars with and without chemical control of the aphid. Data gathered during the 1998 season with the cultivar 'Limpopo', showed an average yield of 2.11 and 1.97 tons/ha for sprayed (one foliar application of metasystox + parathion; see Nel *et al.*, 1999) and untreated plots, respectively (ARC-SGI, unpublished data). These figures compare favourably with the ones obtained with the 1998 mycoinsecticide trial reported here, *i.e.*, 2.17 and 1.98 tons/ha for fungus-treated and control plots, respectively. Moreover, although this difference in yield (fungus-treated versus control) was not statistically significant (P = 0.2153), the slightly higher yield obtained with the fungus-treated plots amounted to *ca.* R150/ha given an average wheat market value of R810/ton during that season. By using mean-separation analyses Nault & Kennedy (1998) also found a weak relationship between crop yield and defoliation by *Leptinotarsa decemlineata* (Say) on potatoes, suggesting that these procedures do not have sufficient statsitical power to detect small reductions in yield. A performance comparison between the mycoinsecticide and chemical insecticide applied to cultivar 'Elands' in 1999 was not possible because of feeding damage inflicted by the red-billed quelea, *Quelea quelea* (L.) (Aves, Ploceidae), in chemical plots during 1999 (Vicki Tolmay, ARC-SGI, personal comm.). However, late-season aphid populations were very low in 1999, and the mycoinsecticide treatments had no detectable effect on yield.

Russian wheat aphid-resistant cultivars differ in their ability to retain their yield potential under severe *D. noxia* infestations. Tolmay (2002) demonstrated this intrinsic genotypic variation among fifteen resistant cultivars by comparing the yields of 'aphid-free' plots with those of severely infested plots over a two-year period. Yield retention (expressed as a percentage of the 'aphid-free' control) varied, on average, from only 60.5 to 117%. Obviously, aphid control in years when high *D. noxia* infestations occur may well be economically justifiable on resistant cultivars with the lower levels of resistance. However, given the integration of other biological control agents such as predators and parasitoids (Marasas *et al.*, 1997), chemical intervention under these circumstances could jeopardise the long-term objectives of the integrated strategy being developed by ARC-SGI. Recent successes with the introduction (Prinsloo, 1998) and establishment (Prinsloo *et al.*, 2002) of an exotic aphelinid parasitoid, *Aphelinus hordei* Kurdjumov, underscore these concerns.

The rainfall events experienced during 1999 resulted in very low aphid densities after 99/10/18. Densities averaged only 0.77 aphids per plant during the following three survey dates, effectively dissembling treatment effects also in grain yield. Similar 'knock-down' effects due to rainfall have been reported for *D. noxia* feeding on susceptible wheat (Kriel *et al.*, 1984, 1986). However, on susceptible wheat, aphid populations often showed a dramatic increase after such events (Kriel *et al.*, 1984, 1986), a phenomenon ascribed, in part, to the improvement in nutrient status of the host plant (Kriel *et al.*, 1986). A similar aphid resurgence was not observed on the antibiotic host plant, suggesting that natural weather events may play an important role in regulating *D. noxia* populations on such cultivars. In fact, the open leaf architecture of resistant cultivars may augment the physical impact of rainfall on resident aphid populations.

Although the Mycotrol® ES application was administered a mere eight hours prior to the onset of rain showers on 99/10/20, >60% control was observed nine days later. Perhaps the oil carrier and 0.1% rate of surfactant used helped to retain conidia on aphid and plant surfaces under these conditions. In addition, higher humidity and low solar irradiation due to cloudy conditions (only 4 hours of total irradiation measured for 99/10/20 and 99/10/21; ARC-ISCW, Report no. 19833) may have supported conidial survival (Morley-Davies *et al.*, 1995) and subsequent infection. Also, *B. bassiana* strain GHA has been reported to retain 90% conidial viability for many months when stored at 5°C – 25°C (Jaronski, 1997). Temperatures were low (mean 14.1°C) and thus highly favourable to *B. bassiana* survival during the initial 72h post application.

Interestingly, James *et al.* (1998) reported the highest mortality of a coccinellid beetle at between 14°C and 20°C for *B. bassiana*. On the other hand, control mortality was lowest at 20°C – 28°C and increased at temperatures above and below this range, indicating that both the high and low temperatures tested were stressful for the insects. Although *D. noxia* has a relatively low temperature threshold (Aalbersberg *et al.*, 1987), a similar indirect synergism between *B. bassiana* and low temperatures (acting as a stress factor) cannot be excluded here. In this regard, low temperatures may also slow the development of *D. noxia*, resulting in higher susceptibility to fungal infection due to the lengthening of time between ecdyses or inhibition of host immune response (James *et al.*, 1998).

6.5 Current review

Observations from this study suggest that secondary pick-up of fungal inoculum may play an important role in the timing of mycoinsecticide applications against *D. noxia*. In this regard, migration of *D. noxia* onto the flag leaves should be further investigated as a behavorial trait for possible exploitation when considering the use of a mycoinsecticide. The concept of increased insect movement leading to enhanced secondary pick-up of fungal inoculum is supported by various researchers (Griffiths & Pickett, 1980; Furlong & Pell, 1996; Shah *et al.*, 1999; Roditakis *et al.*, 2000). However, genotypic variation among cultivars will most likely affect the level of aphid activity, and hence, the level of control following an early application of the fungus. On the other hand, an application of the fungus during the early flag-leaf stage may result in a more reliable level of control, as was observed during both 1998 and 1999 using different aphid-resistant cultivars.

The economic feasibility of aphid control on resistant cultivars will eventually be determined not only by the impact of *D. noxia, per se*, but rather the collective impact of at least six cereal-aphid species (Annecke & Moran, 1982). Field studies by Schotzko & Bosque-Pérez (2000) indicated no significant effect on densities of three other cereal-aphid species with the *D. noxia*-resistant genotype PI 294994 (cultivar 'IDO-488'). In fact, during their study, densities of *Metopolophium dirhodum* (Walker), *Rhopalosiphum padi* (L.), and *Sitobion avenae* (F.) reached levels of 55, 19.5, and 18.3 aphids per tiller, respectively. The local trend in reduced chemical intervention (Marasas, 1999) may, therefore, lead to a resurgence of other aphid pest species such as *Schizaphis graminum* (Rondani) and *R. padi*, previously contained as a result of extensive *D. noxia*-control initiatives (Rabe *et al.*, 1989).

These trials have demonstrated the potential integration of a mycoinsecticide for aphid control on resistant wheat. Issues in need of further investigation include economic threshold levels for individual resistant cultivars, large scale field application (tractor and/or aerial), and development of local mycoinsecticide products with high efficacy towards not only *D. noxia*, but also the other five cereal-aphid species attacking wheat in South Africa. This last research issue is presently being investigated at ARC-SGI (Hatting, 2001).

CHAPTER 7

DESCRIPTION OF A STANDARD BIOASSAY METHODOLOGY FOR SCREENING ENTOMOPATHOGENIC HYPHOMYCETES AGAINST CEREAL APHIDS

7.1 Introduction

Following the discovery of the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov) in the USA during the mid-1980's (Stoetzel, 1987; Morrison *et al.*, 1988), several entomophthoralean, as well as hyphomycetous fungi, were bioassayed in attempts to characterize their virulence against this aphid (Feng & Johnson, 1990; Feng *et al.*, 1990b; Feng & Johnson, 1991; Vandenberg, 1996; Vandenberg *et al.*, 1998). Also, in Europe, a strain of the hyphomycete *Paecilomyces fumosoroseus* (Wize) Brown & Smith, was assayed against *D. noxia* (Mesquita *et al.*, 1996). The bioassay methodologies used in these studies differed significantly, and this factor, in addition to differences in fungal strains, most probably contributed to the large variation in terms of LC_{50} values reported, *e.g.*, 46 – 56 conidia / cm^2 (Vandenberg, 1996) versus 1 780 conidia / cm^2 (Mesquita *et al.*, 1996) for *P. fumosoroseus* against *D. noxia*. Reasons for conflicting LC_{50} values obtained during bioassays of this nature include inoculation technique and equipment, different host plant varieties, source insect populations, carrier agents, formulation, insect age/stage, post-inoculation incubation, method of mortality assessment and interpretation, assay design, and statistical analyses (Vandenberg, 1996; Butt & Goettel, 2000). Unless these parameters are standardised, direct comparison between bioassay studies (*i.e.*, LC_{50} and/or LT_{50} values) is impossible.

In view of the above-mentioned discrepancies, the choice of bioassay design is not an easy one. It is especially important that the objective of a bioassay be clearly defined before a protocol is adopted. According to Butt & Goettel (2000), bioassays of entomopathogenic fungi are used in one or more of the following applications: (1) determination of virulence, (2) comparison of virulence among isolates, (3) determination of host range, (4) determination of epizootic potential, and (5) studies on the effects of biotic and abiotic factors such as host age, host plant, temperature, humidity and formulation. Overall, bioassay precision is another important issue when deciding on a given protocol or design for testing entomopathogenic microbes. This can only be measured by the reproducibility of results in a series of identical experiments (Burges & Thomson, 1971).

Considering the above-mentioned, three main objectives were formulated regarding the use of entomopathogenic fungi as part of the *D. noxia* integrated-control programme being developed at the Small Grain Institute (SGI) of the Agricultural Research Council (ARC) of South Africa. Objectives were (1) to compare the virulence of several isolates of the Hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin to *D. noxia*, (2) to investigate the effect of antibiotic host-plant resistance on *B. bassiana*-induced mortality of *D. noxia* and, (3) to screen hyphomycetous fungi against other important cereal aphid species such as *Rhopalosiphum padi* (L.) and *Schizaphis graminum* (Rondani). As these objectives entailed the use of a laboratory bioassay, a single assay design was sought in order to accommodate all three studies. Ideally, this methodology should aim to:

- maintain aphids on a live host-plant system (tritrophic interaction studies),
- limit control mortality by reducing aphid handling / transfers,
- provide a means for accurate dose administration and quantification,
- accommodate the use of different cereal-aphid species,
- confine confidence intervals, and
- ensure good bioassay reproducibility.

Following is a description of the bioassay protocol developed for use in the Bioinsecticide Research Laboratory of ARC-SGI. Photographs depicting selected steps of the protocol support this section.

7.2 Material and methods

7.2.1 Preparing aphids

The insect host, against which the pathogen is to be assayed, is in itself a biotic variable and should be standardized in terms of life stage, age, and overall physiological condition (Milner & Soper, 1981; Boucias *et al.*, 1984; Lizen *et al.*, 1985; Vandenberg, 1996). Another factor for consideration during host standardization is the genotypic disposition of parthenogenetically reproducing insects. Aphid reproduction encompasses obligate and facultative apomictic parthenogenesis and holocycly (an annual sexual phase) (Hales *et al.*, 1997).

Individuals produced by apomictic parthenogenesis are essentially clones as no meiotic division occurred, resulting in the diploid progeny having the same genetic constitution as the genitor (Chapman, 1969). Subsequently, an aphid culture was initiated by introducing a single *D. noxia* female (collected at ARC-SGI; grid reference S28°09.492'/E28°18.312' on 02/03/20 from rescue grass, *Bromus catharticus* Vahl) into a cage of wheat (cultivar 'Betta'). This clone was designated SGI2021 and maintained in the greenhouse at 25°C, natural light and 50 ± 5% RH.

In order to obtain one-day-old adult apterae, a slightly modified method to that used by Vandenberg (1996) was adopted. This method entailed confining a known number of adult aphids (clone SGI2021) on potted wheat seedlings (cultivar 'Betta', growth stage 13; see Tottman, 1987) for 24 hours. Adults were then eliminated by crushing with forceps, and nymphs were reared at 25°C, natural light and 50 ± 5% RH, until the adult stage (*ca.* eight days).

7.2.2 Inoculation of aphids

7.2.2.1 Collecting aphids

Adult aphids were collected by gently breathing warm air over a pot with seedlings and resident aphids, as it was held at an angle over a sheet of white paper. This technique resulted in the dislodging of aphids without physical contact and thus less chance of injury due to stylet damage. Free-roaming adults (stage identification according to Aalbersberg *et al.*, 1987) were then collected by means of a fine camel-hair paint brush, and groups of 20 aphids were placed separately into 0.5ml Eppendorf® microtest tubes (Merck NT Laboratory Supplies (Pty) Ltd. Bloemfontein, South Africa. Cat. no. 306/0420/02).

In order to reduce mobility of aphids during inoculation, the holding tubes (laid horizontally) were kept in the dark for 6-7 hours at 5°C prior to the assays. Prior to loading of the tubes with aphids, a small hole (*ca.* 900 µm diameter) was pierced into the cap of each tube with a hot needle in order to regulate air pressure and allow gas exchange.

7.2.2.2 Preparing inoculation platforms

Triangles with 30mm sides were cut from no. 4 Whatman® filter paper (Merck NT Laboratory Supplies (Pty) Ltd. Bloemfontein, South Africa. Cat. no. 234/0320/04) and submerged in *ca.* 20ml of a 0.01% nonionic organosilicone surfactant (Break-Thru®; Goldschmidt Chemical Corporation, Hopewell, VA, USA) solution inside a 90mm Petri dish (Fig. 7.1). Saturated triangles were removed from the solution using forceps and placed flat onto another disc of dry filter paper, which served to absorb excess water. The moist triangle was then lifted from the filter-paper disc and placed in a Petri dish containing a fine hypoallergenic talc (Johnson's Baby Powder; Johnson & Johnson®, East London, South Africa) which adhered to the undersurface of the triangle. Using forceps, the triangle was gently pressed onto the talc to ensure adequate coverage. The talc clogs the aphids' tarsi and therefore inhibits them from moving to the underside of the triangle.

The triangle was then removed from the dish and positioned horizontally by securing the centre of one side in a 20mm alligator clip (AC/DC Dynamics, Driehoek, Germiston, Johannesburg, South Africa) glued to a 25mm steel nail at an angle of 90°. The nail with platform was inserted into a ball of adhesive glue (Prestic®; Permoseal (Pty) Ltd., Beverley Roads, Montague Gardens, Cape Town, South Africa) positioned on a predesignated circle (18cm-diameter), drawn on the turntable of the spray tower, to minimize dose variation due to possible deposition differences in the radial dimension (Fig. 7.2).

7.2.2.3 Spray apparatus

All spray applications were administered using a precision spray tower constructed after Burgerjon (1956) (construction by Faculty of Science Workshop, University of Natal, Pietermaritzburg, South Africa; Fig 7.3). The Burgerjon tower is a closed system which is calibrated with great precision.

The spray tower was fitted with an air-atomizing nozzle (Fluid Cap 2850 + Air Cap 67147-ENP) mounted in a ¼ J nozzle body (Spraying Systems Co., Wheaton, IL). The nozzle was connected to a regulator valve providing a constant airflow of 5 litre/min. The base of the Burgerjon spray tower comprises a unique rotating disc or turntable (25cm diameter; 33 rpm) on which the triangles with resident aphids were positioned during inoculation.

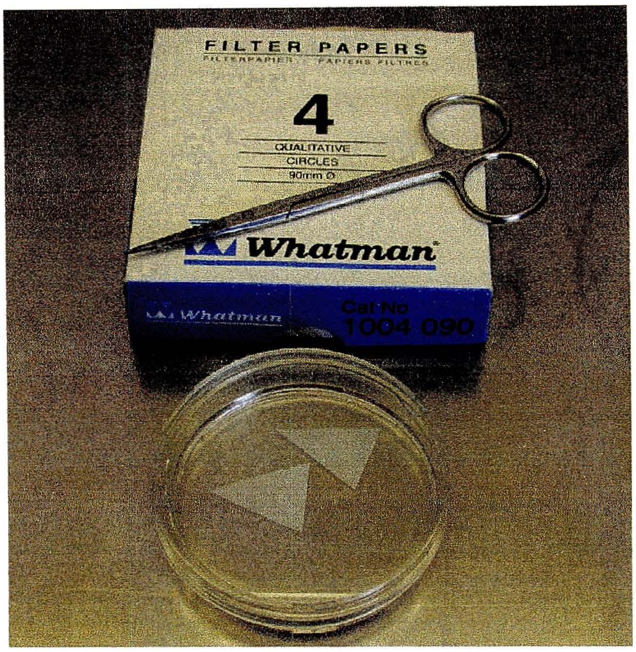


Figure 7.1. Saturation of triangles in 0.01% aqueous Break-Thru® solution

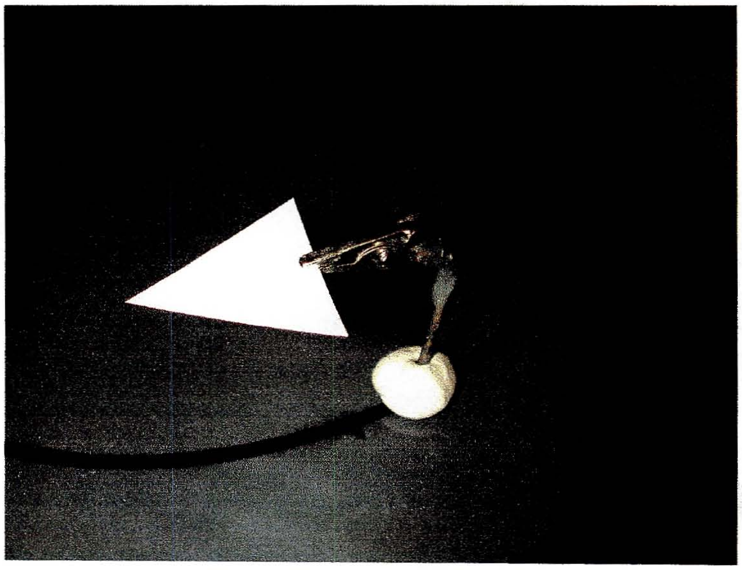


Figure 7.2. Triangle held horizontally on spray tower turntable

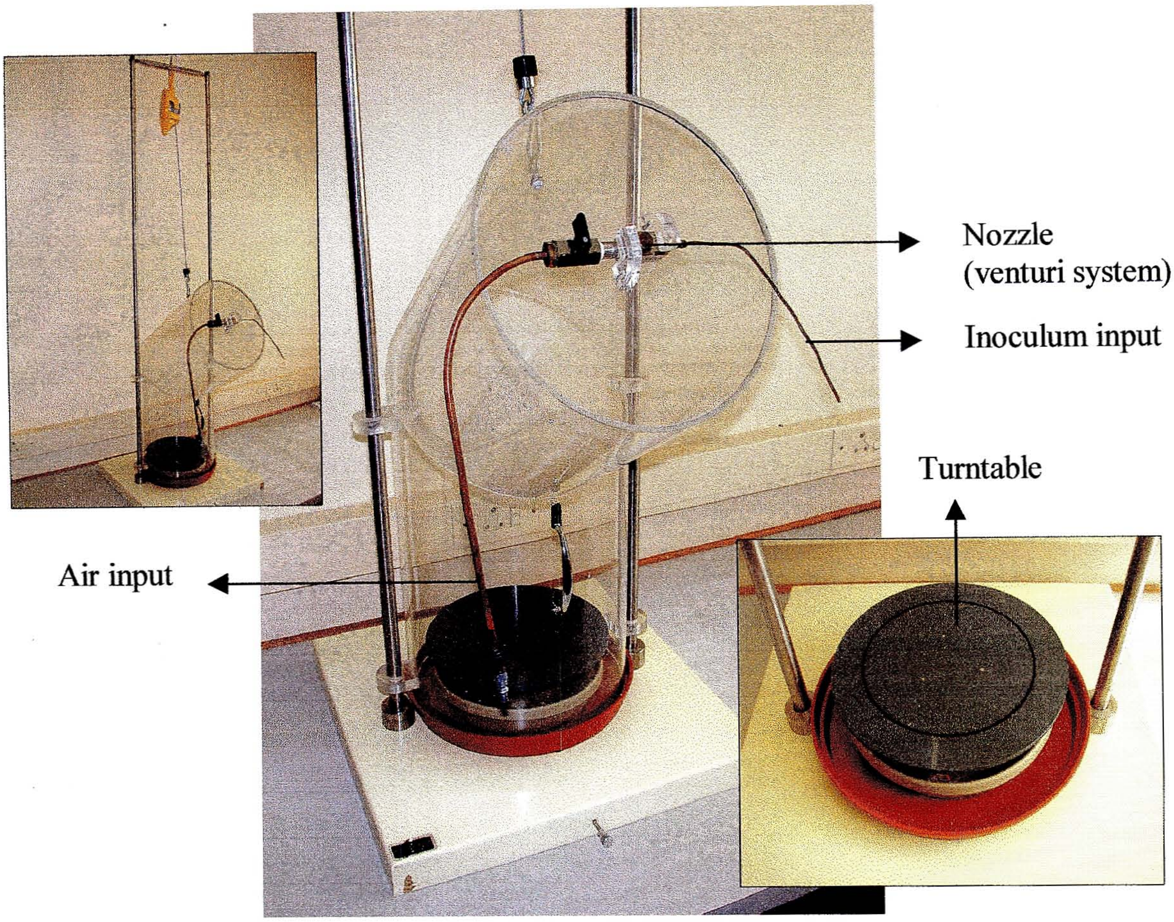


Figure 7.3. Burgerjon Spray Tower

7.2.2.4 Inoculating aphids

Aphids were gently 'poured' onto the triangle from the Eppendorf® tube, and each aphid was then positioned (using a fine camel-hair paintbrush) so as not to mask or hinder another insect from being adequately inoculated during spraying (Fig. 7.4). Aphids were promptly sprayed taking advantage of their lethargic behaviour following cold treatment. In the event of aphid movement on the triangle prior to and/or during spraying, the talc assisted in preventing aphids from moving to the underside of the triangle (due to clogging of the tarsi) thereby ensuring inoculation of all test insects. Generally, however, most aphids stopped moving as soon as the spray application was administered. Dose was quantified by counting the number of conidia deposited on a 7cm-diameter plate of 2% water agar placed adjacent to and on the same horizontal level as the triangle. Conidial counts were executed according to the technique devised by Wraight *et al.* (1998) and dose expressed as the number of conidia per square millimetre. The average of six sample areas was used in the analyses.

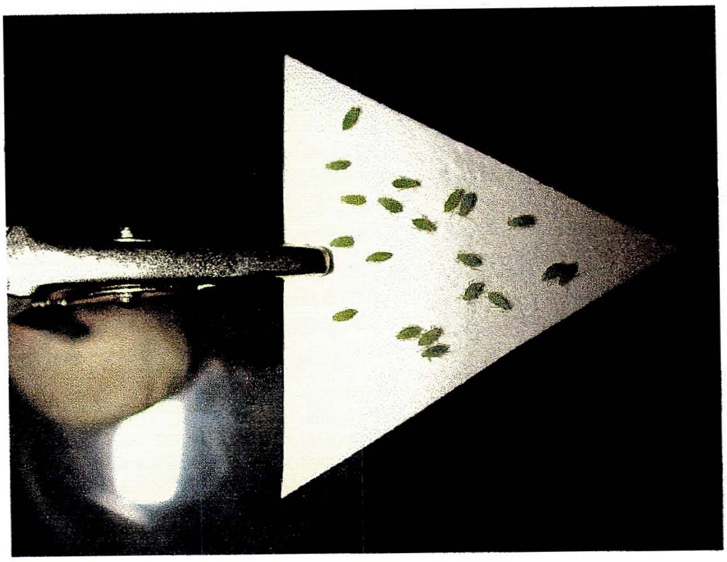


Figure 7.4. Aphids positioned to ensure adequate inoculation

7.2.3 Construction and preparation of rearing units

Specially designed rearing units were constructed to maintain treated aphids on actively growing wheat seedlings (cultivar 'Betta'; growth stage 20). These units had to support healthy plant growth, ensure easy and uniform watering of plants, and facilitate daily observation and removal of dead aphids, while preventing aphid escape. The last criterion was especially important in view of aphid losses previously reported with other live-plant aphid-rearing systems (Yeo, 2000; see also Chapter 8).

Each rearing unit comprised three basic structures, (1) a 350ml, 100mm-diameter pot (PC Plastics CC., Robbertville, Roodepoort, South Africa), (2) a 20mm-wide tapered plastic collar with diameters of 85 and 93mm, and (3) a 1500ml, 90mm-diameter plastic bottle (Hilfort Plastics (Pty) Ltd., Stikland, Cape Town, South Africa. Cat. no. 1-194-SCR-NAT-01) acting as a cage after being inverted over the seedlings with resident aphids. Both cage and collar were constructed from the same bottle. The collar was glued onto the inside rim of the pot with the 85mm diameter opening at the top (Fig. 7.5). This allowed for the cage to be inverted over the outside of the collar effectively sealing the cage onto the collar due to the slight angle of contact between the two structures (Fig. 7.6). Three seeds were sown in a group (off-centre in the pot) to ensure contact between the seedlings for easy aphid movement between plants. In preliminary assays fewer than three plants, of younger age per pot, became severely stressed due to aphid feeding and often did not survive the duration of the assay.

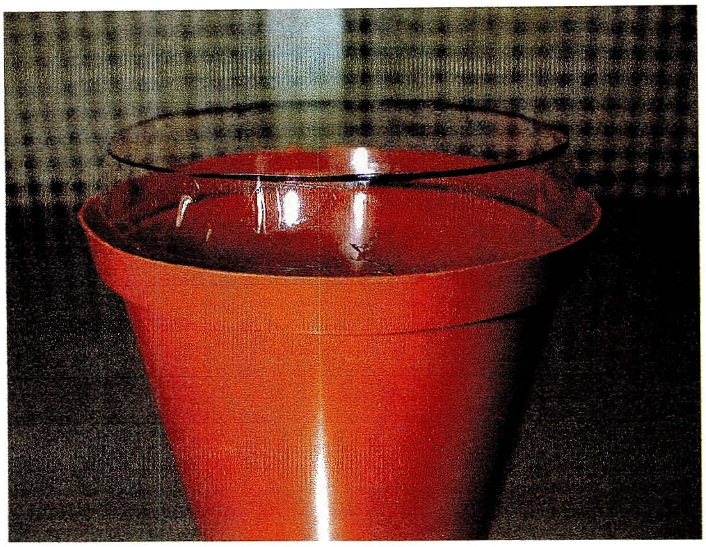


Figure 7.5. Tapering collar glued onto the inside rim of the pot

Figure 7.6. Cage later inverted over the outside of the collar, effectively sealing the two structures onto each other

Directly prior to the assay, a thin layer of fine sterile sand (≤ 400µm diameter particles) was strewn onto the surface of each pot to assist later in finding dead aphids inside the rearing unit. Leaves were trimmed to *ca.* 50mm lengths to ensure easy coverage of plants with the cage (Fig. 7.7). During the assay, individually caged pots (with three 8mm-diameter holes in the bottom) were held inside a 500ml plastic dish. By pouring the desired quantity of water directly into the dish, all units were easily and uniformly watered without removing the cage and disturbing the layer of fine sand on the surface of the soil.

Figure 7.7. Fine sand added and leaves trimmed to *ca.* 50mm lengths for easy coverage with the inverted cage

7.2.4 Transfer of aphids to plants post inoculation

Immediately after spraying, the nail was removed from the glue and inserted into the sand so as to allow the opposite corner and sides of the triangle to make contact with the seedlings (Fig. 7.8). Because of isolated cases of aphids dropping from the triangle during the inoculation and transfer (from spray tower to pot) process, the actual number of aphids on the triangle was again determined. A 90mm-diameter black-painted plastic cage (see 7.2.3) with 50mm x 90mm clear window on one side, was then inverted over the seedlings and triangular platform. The cage was positioned with the clear window directly opposite the platform resulting in aphids being attracted onto the seedlings by the light (aphids acting positively phototactic) and plant-volatile cues (Fig. 7.9). In order to ensure saturated humidity conditions, the inside of the (non-ventilated) bottle was moistened with a hand-held sprayer prior to covering of the seedlings.

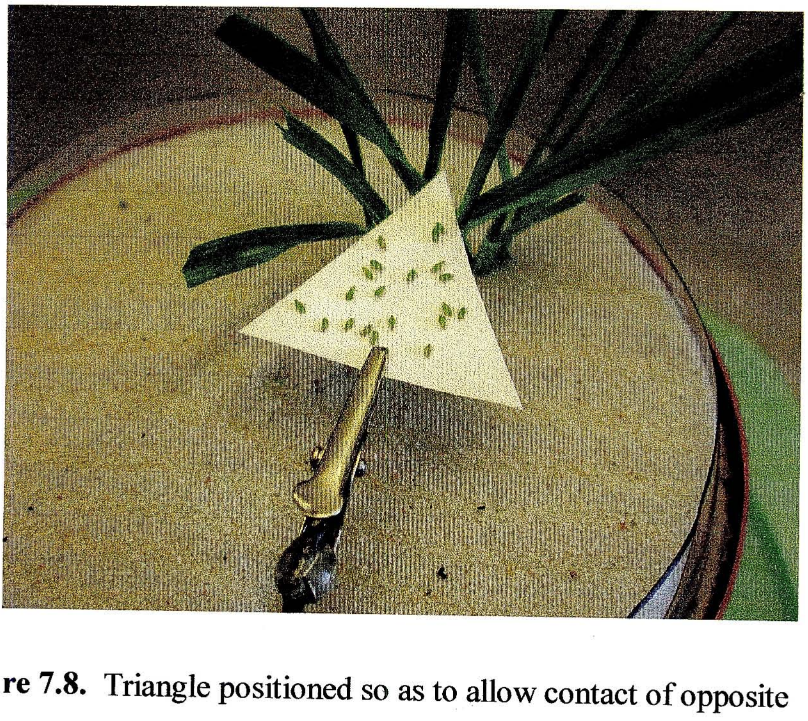


Figure 7.8. Triangle positioned so as to allow contact of opposite corner and sides with seedlings

Figure 7.9. Black bottle positioned with clear window directly opposite triangle resulting in aphids being drawn onto the seedlings by the light and plant-volatile cues

7.2.5 Incubation and mortality assessment of inoculated aphids

Caged seedlings were incubated under saturated humidity conditions for 24 hours at 25°C, continuous light. This time period allowed for aphid movement onto the seedlings and facilitated spore germination. After 24 hours, the black cage and nail with platform were removed and seedlings covered with a 90mm-diameter clear plastic cage (see 7.2.3) with a 50mm x 30mm ventilated opening (465μm gauze size) on either side. In order to prevent a humidity gradient from developing inside the unit, five 6mm-diameter holes (covered with 465μm gauze) were made in the top of each cage (Fig. 7.10).

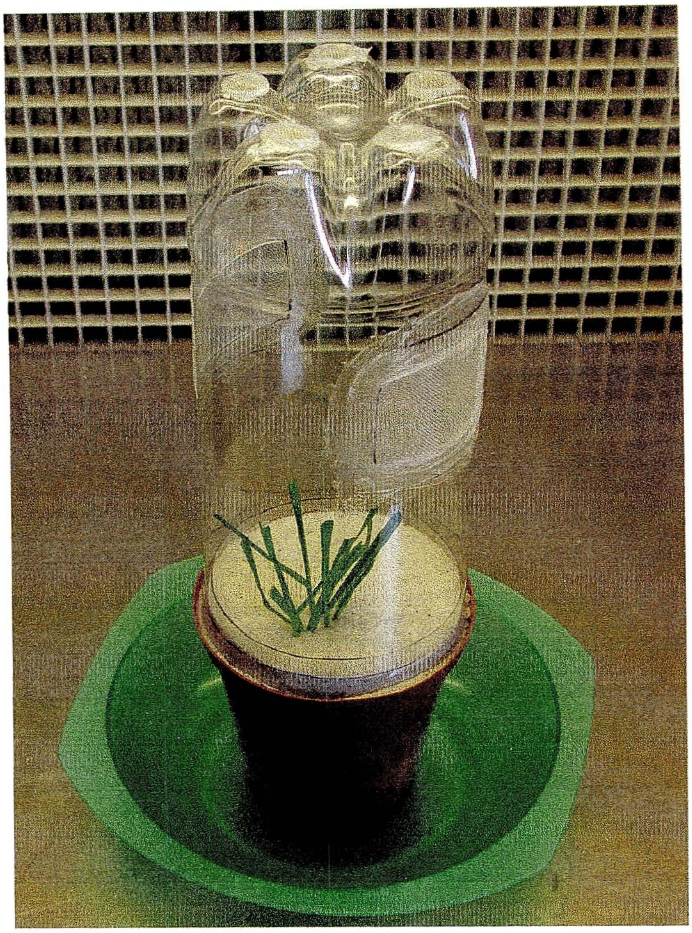


Figure 7.10. Ventilated cage inverted over seedlings with resident aphids

Dead aphids found immediately after the initial 24-hour period were considered handling losses and removed from the rearing unit. Adjustments to the total initial number of aphids were made accordingly. Seedlings with resident aphids were maintained under greenhouse conditions at 25 ± 2°C, 40 ± 5% RH, natural light, for the 5-day duration of the assay.

Although nymphs were produced during the course of the assay, development only up to the 4th instar was possible under the unique conditions of the assay over this time period (Hatting, unpublished observations; see also Aalbersberg *et al.*, 1987). Rearing units were inspected daily for the presence of dead adult aphids. Dead aphids were collected and placed on 2% water agar and incubated for 72 hours at 25°C for verification of (overt) mycoses.

7.3 Results

This protocol was used in screening (single-dose assay) four strains of *B. bassiana* against *D. noxia* and to establish virulence parameters (multiple-dose assays), expressed as LC_{50} values, of the best isolate. These results are reported in Chapter 8.

7.4 Discussion

Previously published protocols used to measure and compare virulence (LC_{50}'s) of entomopathogenic fungi against *D. noxia* have not provided for the post-inoculative incubation of aphids on live host plants. This, however, is imperative when investigating the tritrophic interaction of host plant, pest and pathogen (Hsiao & Khachatourians, 1997b; Knudsen & Wang, 1998; Poprawski *et al.*, 2000). Moreover, Yeo (2000) evaluated three incubation systems for rearing the black bean aphid, *Aphis fabae* Scopoli, post inoculation. The three methods included (1) excised leaves set in 2% water agar in 9cm Petri dishes, (2) a Blackman box (12.5 x 8 x 2cm) with muslin-covered ventilation hole (excised leaves held in water-saturated foam), and (3) a live plant under a lampglass covered with muslin. That author concluded that, "*Generally, those aphids incubated on whole plants survived better than those in Petri dishes, which in turn survived better than those incubated in Blackman boxes*". These results are not surprising, considering the superior nutritional status of a live host plant (Chandler, 1997), coupled to the fact that aphids are essentially phloem-feeders (Tjallingii, 1978; Srivastava, 1987).

In previous assays with Hyphomycetes against *D. noxia*, two methods of inoculating the test insects were used. These methods entailed dipping or immersing insects in spore suspensions of known concentration (Feng *et al.*, 1990b; Feng & Johnson, 1990) and topical spray applications using high-tech precision spray towers (Mesquita *et al.*, 1996; Vandenberg, 1996; Vandenberg *et al.*, 1998).

Yokomi & Gottwald (1988) found that these two techniques were equally effective in delivering conidia of *Verticillium lecanii* (Zimmermann) Viégas to *Myzus persicae* (Sulzer). However, slightly lower variation in LC_{50} values was obtained with the spray technique ($r^2 = 0.80$; $LC_{50} = 1.65 \times 10^6$ conidia per ml) compared with the immersion technique ($r^2 = 0.73$; $LC_{50} = 1.48 \times 10^6$ conidia per ml). Ultimately, these authors opted to use the spray technique in subsequent assays as this technique better simulated field procedures.

A disadvantage of the immersion technique can be high control mortalities as previously reported for aphids (Chandler, 1997) as well as for coleopteran larvae (Doberski, 1981). Regarding *D. noxia,* however, no indication of the actual percentage control mortality was reported following immersion of insects in the (inoculum-free) solution only (Feng & Johnson, 1990; Feng *et al*., 1990b).

Dose quantification (expressed as the number of conidia retained per test insect) may not be very accurate following immersion of insects in spore suspensions, even of known concentration. Counting the number of colony-forming units (following maceration of insects and plating onto a selective medium) will not differentiate between infectious conidia on the cuticle and conidia contained within the suspension liquid on the host which were unlikely to infect (Chandler, 1997). Also, techniques aimed at washing the conidia from the cuticle of treated insects (Doberski, 1981) cannot guarantee complete removal of all conidia, thus leading to a possible underestimation of dose parameters. Although direct counting of the number of conidia attached to the insect cuticle is possible by means of fluorescence microscopy (Chandler *et al*., 1993; Butt, 1997), this can be an extremely labour-intensive and time-consuming exercise.

Considering the topical spray technique, aphids are known to acquire fungal inoculum simply by moving over treated leaf surfaces (Hall, 1979; Roditakis *et al*., 2000). Therefore, aphid movement within a bioassay system where a topical spray application is administered to the aphids and food substrate, simultaneously, could result in additional inoculum being acquired. The respective LC_{50} values of only 0.46 and 0.91 conidia per square millimetre, reported with *P. fumosoroseus* and *B. bassiana* against *D. noxia* (Vandenberg, 1996), suggest considerable aphid movement within the bioassay system resulting in additional conidia being acquired by the test insects. Because the factors that affect the general restlessness of aphids following introduction into assay chambers are not known and thus uncontrollable, this phenomenon could contribute to the heterogeneity of assay results.

In view of above-discussed problems, an important objective of the bioassay design described herein was treatment of aphids with a topical spray application of conidia quantifiable on a per-unit surface-area basis, with minimal secondary acquisition of conidia. Aphids were thus treated on a non-leaf substrate and then encouraged to move immediately onto untreated plants, where they remained for the duration of the assay. Manual transfer of *D. noxia* post inoculation (Feng & Johnson, 1990; Vandenberg, 1996; Mesquita *et al*., 1996) may lead to additional stress and/or physical injury. However, the protocol described in this chapter addresses this problem by exploiting the positively phototactic nature of *D. noxia* (Hatting, unpublished observations) and general aphid response to plant-volatile cues (Anderson & Bromley, 1987). To further limit secondary pick-up of conidia, the substrate for use as inoculation platforms had to allow unhindered aphid movement while acting as a sponge to draw surface-deposited inoculum away from the insects. The Burgerjon spray tower atomises the droplets resulting in a very fine mist being deposited on the tower turntable (Burgerjon, 1956). Although the main purpose of moistening the filter paper was to assist with retention of talc to repel aphids from the underside of the triangle, a cohesion force between water molecules in the paper and spray droplet was also established. This force acts to rapidly draw the droplets into the filter paper, thereby trapping the conidia in and/or on the fibres of the paper. These features would lessen the chance of aphids coming into contact with conidia post inoculation.

7.5 Current review

Although certain aspects of the bioassay protocol described here do reflect conditions likely to be encountered in the field (*e.g.*, topical spray application and a live host-plant substrate) caution should be exercised when extrapolating results obtained during laboratory bioassays with aphids to the field situation. Conflicting results so obtained have been (hypothetically) explained by differences in aphid species behaviour (Sopp *et al.*, 1989 and references within) and environmental conditions (Dorschner *et al.*, 1991). Therefore, the design developed here is aimed rather at a standard, nonbiased comparison of virulence between isolates within the framework of accurate dose administration and quantification, minimal control mortality, and good bioassay reproducibility (precision).

Moreover, the live host-plant system will facilitate tritrophic interaction studies while the use of cereal aphid species other than *D. noxia* can also be accommodated with this design. Another measurable phenomenon is cadaver distribution. Although at least some aphid cadavers infected by *B. bassiana* are known to be attached to the plant substrate by means of the proboscis (see Chapter 4; Feng *et al.*, 1990a), the ratio of cadaver location on the plant substrate compared with sites other than the plant (*e.g.*, soil surface) remains unknown. The described method will answer this. This is especially important in view of mortality assessments when conducting field trials with Hyphomycetes against aphids. Also, an investigation into pre-mortem behaviour of infected insects is possible. Such changes may include an early cessation in feeding behaviour (Müller, 2000), and hence, an immediate level of control.

CHAPTER 8

EVALUATION OF A BIOASSAY METHODOLOGY FOR SCREENING *Beauveria bassiana* AGAINST RUSSIAN WHEAT APHID (HOMOPTERA: APHIDIDAE)

8.1 Introduction

The Small Grain Institute (SGI) of the South African Agricultural Research Council (ARC) has acquired several indigenous and exotic strains of the entomopathogenic hyphomycete, *Beauveria bassiana* (Balsamo) Vuillemin. The rationale behind these research endeavours has been the possible development of a myco-insecticide for inclusion in an integrated-control programme against the Russian wheat aphid, *Diuraphis noxia* (Kurdjumov), and other secondary cereal-aphid species (Hatting, 2001; see also Chapter 6).

An important biological trait when selecting isolates for development as myco-insecticides is their virulence towards the target insect pest/s. According to Prior (1992), the term virulence can be defined as "*a quantitative measure of the capacity of an individual pathogen genotype to cause infection*". This capacity can only be measured through the use of a standardized bioassay methodology, the efficacy of which is reflected by the reproducibility of results in a series of identical experiments (Burges & Thompson, 1971).

As discussed in Chapter 7, several practicalities have to be considered when deciding on the use of a given aphid-bioassay design. The most important features of the protocol proposed in Chapter 7 entail the use of a live host-plant system and restriction of secondary dose acquisition post inoculation (*i.e.*, accurate dose quantification).

The purpose of this study was to evaluate the bioassay protocol described in Chapter 7 in terms of precision (between-assay variability) and overall practical feasibility. Initially, four strains of *B. bassiana* were chosen, based on percentage germination and sporulation intensity, and then assayed in a single-dose maximum-challenge test. The best-performing strain (based on percentage mortality and mean mortality time) was then used in two independent series of five multiple-dose assays each to establish LC_{50} parameters for that specific strain.

8.2 Material and methods

8.2.1 Aphids

See Chapter 7, section 7.2.1.

8.2.2 Fungal isolates

To date, 16 indigenous and 5 exotic strains of *B. bassiana* have been acquired by ARC-SGI (Table 8.1) for evaluation and possible development as biorational insecticide/s. Four exotic strains were acquired within the framework of a bilateral research agreement between the Agricultural Research Council of South Africa and the Hungarian Academy of Sciences (South African National Research Foundation Grant number 2045313). A commercial strain of *B. bassiana* (strain GHA), developed by Mycotech Corp., Butte, Montana, USA, was imported to South Africa as an emulsifiable suspension formulation (*i.e.*, Mycotrol® ES) and an unformulated technical powder during 1997 and 2000, respectively. Although some *B. bassiana* isolates were collected from nonaphid hosts, most were reisolated from *D. noxia* infected in the laboratory (Table 8.1).

8.2.3 Fungal preparations

Isolates were cultured on Sabouraud dextrose agar (Biolab Diagnostics, Midrand, Halfway House, South Africa) supplemented with 1% yeast extract (Difco Laboratories, Detroit, Michigan) (SDAY) at pH 6.7. No antibiotics were added to the medium. Five plates (9cm-diameter Petri dishes) of each culture were incubated for 14 days at 25°C with a photoperiod of 16:8 (L:D) h. Cultures were air-dried inside a laminar-flow hood for 48 hours and the conidia harvested by scraping from the surface with a sterile scalpel. Fungal material so obtained was passed, twice, through a 110μm-gauze sieve to separate conidia and hyphal material. Unformulated (technical) powders were stored in sealed 10ml sterile tubes at approx. -15°C until use in the assays.

8.2.3.1 Single-dose assay

The bioassay methodology used during the initial single-dose assay differed slightly from that described in Chapter 7. Following the single-dose assay, procedures were further refined in order to reduce both the level of control mortality and the number of missing

Table 8.1 Identification of fungal isolates of *Beauveria bassiana* obtained by ARC-SGI

***B. bassiana* isolate[a] (ARSEF[b])**	**Insect host of origin**	**Site and date of origin[c]**	**% Germination**	**Sporulation[h]**
PPRI[d] 6688 (5705)	*Diuraphis noxia* (Homoptera)	Langgewens, Western Cape, South Africa, 1997	50	+
PPRI 6689	*Sitotroga cerealella* (Lepidoptera)	ARC-SGI, Bethlehem, Free State, South Africa, 1997	25	+
PPRI 6690	*Bombix mori* (Lepidoptera)	Bethlehem, Free Sate, South Africa, 1997	35	+
PPRI 7040[e] (6967)	Unidentified tick (PPRI 7165)[f]	Hungary[g]	67	++
PPRI 7041[e] (6968)	*Diuraphis noxia* (Homoptera) (PPRI 7166)[f]	Budapest, Hungary, 2000	60	++
PPRI 7042[e] (6969)	***Diuraphis noxia* (Homoptera) (PPRI 7167)[f]**	**Budapest, Hungary, 2000**	97	+++
PPRI 7043[e] (6970)	***Rhopalosiphum padi* (Homoptera) (PPRI 7168)[f]**	**Hungary[g]**	**98**	++
PPRI 7054 (6971)	*Monopetalotaxis doleriformis* (Lepidoptera) (PPRI 7169)[f]	Clanwilliam, Western Cape, South Africa, 2001	70	+
PPRI 7055 (6972)	*Isturgia exerrariae* (Lepidoptera) (PPRI 7170)[f]	ARC-SGI, Bethlehem, Free Sate, South Africa, 2001	70	++
PPRI 7056 (6973)	*Isturgia exerrariae* (Lepidoptera) (PPRI 7171)[f]	Clanwilliam, Western Cape, South Africa, 2001	66	+
PPRI 7057 (6974)	*Monopetalotaxis doleriformis* (Lepidoptera) (PPRI 7172)[f]	Clanwilliam, Western Cape, South Africa, 1999	90	++
PPRI 7058 (6975)	*Monopetalotaxis doleriformis* (Lepidoptera) (PPRI 7173)[f]	Clanwilliam, Western Cape, South Africa, 2001	62	+
PPRI 7059 (6976)	*Monopetalotaxis doleriformis* (Lepidoptera) (PPRI 7174)[f]	Clanwilliam, Western Cape, South Africa, 2000	35	+
PPRI 7060 (6977)	*Monopetalotaxis doleriformis* (Lepidoptera) (PPRI 7175)[f]	Clanwilliam, Western Cape, South Africa, 2000	70	+++
PPRI 7061 (6978)	*Diuraphis noxia* (Homoptera)	ARC-SGI, Bethlehem, Free State, South Africa, 2001	50	++
PPRI 7062 (6979)	*Diuraphis noxia* (Homoptera)	ARC-SGI, Bethlehem, Free State, South Africa, 2001	30	++
PPRI 7064 (6980)	*Diuraphis noxia* (Homoptera)	ARC-SGI, Bethlehem, Free State, South Africa, 2001	65	++
PPRI 7065 (6981)	***Monopetalotaxis doleriformis*** (Lepidoptera) (PPRI 7176)[f]	**Clanwilliam, Western Cape, South Africa, 2001**	97	+++
PPRI 7068 (6982)	*Molopopterus theae* (Homoptera) (PPRI 7177)[f]	Clanwilliam, Western Cape, South Africa, 1999	95	+
PPRI 7082 (6984)	*Bracharoa dregei* (Lepidoptera) (PPRI 7178)[f]	Clanwilliam, Western Cape, South Africa, 2001	82	+
GHA[e] (6444)	***Diabrotica undecimpunctata* (Coleoptera)**	**Corvallis, Oregon, USA, 1977[i]**	98	+++

[a] Isolates in bold chosen for single-dose assay based on ≥97% germination and profuse sporulation.

[b] Accessioned with the United States Department of Agriculture Agricultural Research Service Collection of Entomopathogenic Fungal Cultures (ARSEF), Ithaca, NY.

[c] Isolated by J.L. Hatting, ARC-SGI, unless otherwise indicated.

[d] Accessioned with the National Collection of Fungi, ARC-Plant Protection Research Institute (PPRI), Pretoria, South Africa.

[e] Imported to South Africa under Act 36 of 1983. Permit number 14/2/2/1(9/97/80) and 14/2/2/1(9/00/56).

[f] Strain passed through and reisolated from *Diuraphis noxia* in the laboratory. New accession number.

[g] Isolated by Dr. Bratek Zoltan, Eotvos University, Budapest, Hungary (no further information available).

[h] Symbol indicates sporulation intensity: + low, ++ medium, +++ high made by visual assessment.

[i] Isolated by Dr. John Vandenberg, USDA-ARS, Ithaca, NY.

individuals (see sections 8.3.1 and 8.4). Subsequently, the following steps in the protocol (*i.e.*, used during the single-dose assay) were identified as potentially problomatic and modifications were made accordingly:

- Aphids were kept overnight (*ca.* 18 hours) in Eppendorf® tubes at 5°C before being used in the assay (*cf.* section 7.2.2.1).
- No collar was included in the rearing unit. The cage (non-ventilated black and ventilated clear) was inverted over the seedlings by pushing the bottom rim of the cage directly into the sand to a depth of *ca.* 5mm (*cf.* section 7.2.3).
- The clear ventilated cage had only two openings, *i.e.*, 50mm x 30mm, one on either side, with no ventilation openings in the top (*cf.* section 7.2.5).
- After the initial 24-hour period, seedlings with resident aphids were maintained under growth-chamber conditions at 25 ± 1°C, 40 ± 5% RH, continuous (artificial gro-lux and normal cool-white flourescence) light, for the 5-day duration of the assay (*cf.* section 7.2.5).

For this maximum-challenge test four strains of *B. bassiana* were compared. For each strain, suspensions containing *ca.* $1x10^8$ conidia per ml were prepared by introducing a given weight of technical powder into 10ml of 0.01% aqueous Break-Thru® (Polyether-polymethylsiloxane-copolymer surfactant; Goldschmidt Chemical Corporation, Hopewell, VA, USA). Conidia were brought into suspension by blending with a hand-held blender (Tornado™, Verimark Holdings (Pty) Ltd., Sandton, Johannesburg, South Africa) at 28 000 rpm for 10 seconds. Actual concentrations of conidia/ml were determined using an Improved Neubauer haemacytometer (Merck NT Laboratory Supplies (Pty) Ltd. Bloemfontein, South Africa. Cat. no. 403/0024/00) and adjustments made by either adding technical powder or solution to the original suspension. Final concentrations were verified by counting three (replicate) samples in the haemacytometer. Three squares ($0.02mm^2$ each) were counted on each occasion. Test insects were sprayed with suspensions (5ml aliquots) within 2 h of preparation.

The choice of strains used in this assay was based on comparative germination percentages and sporulation intensity on SDAY. Percentage germination was assessed 1 day prior to the assay. For each of the 21 strains, *ca.* 1mg of technical powder was collected from the holding tube and suspended in 1ml of 0.01% aqueous Break-Thru®, inside a 2ml

Eppendorf® Safe-lock tube (Merck NT Laboratory Supplies (Pty) Ltd. Bloemfontein, South Africa. Cat. no. 306/0314/02). Tubes were shaken vigorously by wrist action for one minute and the contents then poured onto a plate of SDAY. The surface of the medium was inoculated by a swirling motion and excess liquid discarded. Plates were sealed with parafilm and incubated for 24 h at 25°C. Conidial germination was scored by observing 100 conidia at 400X magnification under a light microscope in each of two different fields of view (Table 8.1). Only isolates with an average germination of $\geq 97\%$ were considered for inclusion in the assay. In combination with this criterion, a visual estimation of conidia production was made following culture and harvesting procedures described under section 8.2.3. Based on these combined observations, four strains were identified for use in this assay (see Table 8.1).

Six and seven (replicates) aphid-containing Eppendorf® tubes (20 aphids per tube; see Chapter 7, section 7.2.2.1) were assigned randomly to each treatment group and control, respectively. Treatments were sprayed with 5ml aliquots of conidial suspension, whereas controls were sprayed only with 5ml aliquots of 0.01% Break-Thru®.

8.2.3.2 Multiple-dose assays

The multiple-dose assays were performed according to the procedures described in Chapter 7. The following is an abbreviated description of the methods. A stock suspension containing *ca.* 1×10^8 conidia/ml was prepared by introducing 0.05g of technical powder into 50 ml of 0.01% aqueous Break-Thru® and blending with the Tornado™ blender as previously described. The actual concentration of conidia/ml was determined and adjusted according to procedures described under section 8.2.3.1. A series of six dilutions (dosages) was then prepared from the stock suspension; concentrations ranged from *ca.* 2×10^5 to 1×10^8 conidia/ml. All suspensions were used within 2 h of preparation. Five replicate aphid-containing Eppendorf® tubes (20 aphids per tube; see Chapter 7, section 7.2.2.1) were assigned randomly to each of the six treatment (dose) groups. In testing precision of a protocol, it is important to eliminate other potential sources of variability. In this regard, each replicate batch of insects was treated with spores taken from a single suspension. In this way, variation in the resulting LC_{50} estimates could be attributed to factors other than those potentially associated with different spore suspensions. Treatments were sprayed with 5ml aliquots of conidial suspension. For control comparison, 10 replicates were sprayed with

0.01% Break-Thru®, only. The spray application and conidia quantification methods are described in Chapter 7, section 7.2.2.4. Two independent series of assays were conducted six days apart.

8.2.4 Statistical analyses

8.2.4.1 Single-dose assay

The average numbers of conidia deposited per mm^2 for each treatment were log transformed and analysed by one-way analysis of variance (ANOVA) using the statistical program GenStat for Windows (2000). Treatment means were separated using the Tukey Honestly Significant Difference (HSD) test at the 5% level of significance (Snedecor & Cochran, 1980).

Percentages of aphid mortality were corrected according to Abbott's formula (Abbott, 1925) using the mortality in the untreated control as the correcting factor. Arcsine transformation was applied to mortality data prior to ANOVA with Genstat for Windows (2000). Treatment means were separated using the Tukey Honestly Significant Difference (HSD) test at the 5% level of significance. To establish the mean time of *D. noxia*-mortality within five days post treatment, mortality was calculated for each of the six replicate batches. The number of aphids dying on a given day after treatment was divided by the total mortality after 5 days. This value was then multiplied by the respective day, and values for days 1 - 5 were summed to produce a weighted average time of mortality for each treatment. Log transformation was applied to the average mortality for each day prior to ANOVA with Genstat for Windows (2000). Treatment means were separated using the Tukey Honestly Significant Difference (HSD) test at the 5% level of significance.

8.2.4.2 Multiple-dose assays

Median lethal dose (LC_{50}), fiducial limits and other regression parameters were estimated using the personal computer version of the POLO program developed by Russel *et al.* (1977). Dose responses were corrected for control mortality (Abbott's formula) programmatically. The multiple estimates of LC_{50} from each of the two independent series were log transformed and then compared by standard one-way ANOVA using the statistical program GenStat for Windows (2000). The untransformed regression slopes were similarly compared.

8.3 Results

8.3.1 Single-dose assay

Rates applied per square millimeter are shown in Table 8.2. These rates were considered homogeneous with no significant differences detected between treatments (P = 0.781; Table 8.2). Abbott-corrected percentage mortalities ranged from 71.7% (PPRI 7167) to 97.8% (GHA) (Table 8.2). Compared with the other strains, GHA and PPRI 7168 resulted in > 90% mortality of *D. noxia* (Table 8.2). Considering these two isolates, higher sporulation intensity was observed with GHA (Table 8.1).

Mortality in the surfactant control was high (23.6%), possibly due to the extended period of holding in the tubes prior to the test or to unfavourably high humidity conditions (see 8.2.3.1). Adjustments to the bioassay protocol (see 8.2.3.1 versus Chapter 7) resulted in control mortalities of < 4% measured with the two multiple-dose assays (see 8.3.2). Based on the overall performance of *B. bassiana* strain GHA, this isolate was chosen for use in two multiple-dose assays in order to establish LC_{50} parameters and to investigate between-assay variability.

Table 8.2. Mortality parameters in adult *D. noxia* 6 days after treatment with a single challenging rate of conidia of four isolates of *B. bassiana*

Treatment	No. insects	Application rate [a]	% Mortality[b]	Abbott-corrected % mortality[b]	% Overt mycosis[b]	Mean time (d) of mortality[a]
GHA	117	2139[c] ± 424.5a	98.3 ± 2.7a	97.8 ± 3.4a	96.7 ± 3.6a	2.3 ± 0.6a
PPRI 7168	107	1908 ± 292.4a	93.2 ± 8.4ab	91.0 ± 8.4ab	87.3 ± 11.5ab	2.9 ± 0.7a
PPRI 7176	96	2020 ± 224.4a	79.2 ± 6.4b	72.6 ± 11.0b	68.0 ± 9.8b	3.2 ± 0.6a
PPRI 7167	107	2053 ± 398.4a	78.5 ± 20.6b	71.7 ± 27.1b	66.9 ± 26.3b	2.8 ± 0.8a
Control	120	---	23.6 ± 12.1c	---	---	---

[a] Log 10 transformation applied prior to ANOVA. Means ± standard errors (n = 5) followed by the same letter within the same column are not significantly different by Tukey's HSD test at the 5% level.

[b] Arcsine transformation applied prior to ANOVA. Percentage ± standard errors (n = 6; control n = 7) followed by the same letter within the same column are not significantly different by Tukey's HSD test at the 5% level.

[c] Number ± standard error (n = 6) of conidia per square millimeter.

8.3.2 Multiple-dose assays

The respective rates for the first and second assays ranged from 4 – 1100 and 3 – 1340 conidia per mm^2. Percentage surfactant control mortality was 3.1% and 2.6% for the first and second series, respectively. Regression analysis of probit mortality and log-dosage data of the two series of bioassays are presented in Table 8.3.

Table 8.3. Regression analysis of probit mortality and log-dosage data of ten replicate bioassays of *B. bassiana* strain GHA against *D. noxia* adults

First series (assay no.)	No. insects	LC_{50}[a]	95% FL and CI[a]	Slope	χ^2 (df = 4)
1	110	171	32 – 371	1.75	4.60
2	115	47	7 – 163	1.15	6.83
3	115	46	28 – 76	1.75	2.81
4	112	96	19 – 346	1.30	8.64
5	114	45	5 – 182	1.34	9.86
Mean	---	69 [b]a	33 – 145	1.46 [c] ± 0.12a	---
Second series (assay no.)					
1	118	133	26 – 503	1.18	8.23
2	114	67	36 – 113	1.36	1.85
3	117	82	35 – 146	1.32	3.30
4	104	80	21 – 168	1.69	4.11
5	109	172	16 – 435	1.25	4.37
Mean	---	100 [b]a	61 – 164	1.36 [c] ± 0.09a	---

[a] LC_{50} values and their 95% fiducial limits (FL) and mean LC_{50} values with 95% confidence intervals (CI) expressed as conidia per square millimeter.

[b] Log 10 transformation applied prior to ANOVA. Back-transformed mean LC_{50}s followed by the same letter within the same column are not significantly different by ANOVA at the 5% level (F = 1.32 with 1, 8 df; P = 0.28).

[c] Mean slopes ± standard errors followed by the same letter within the same column are not significantly different by ANOVA at the 5% level (F = 0.42 with 1, 8 df; P = 0.54).

For testing equality of the five probit lines in each series, the POLO program was used to test the hypothesis that both slopes and intercepts (elevations) were equal. These results are presented in Table 8.4.

Table 8.4. Among-assay variability parameters for two independent series of assays of *B. bassiana* strain GHA against *D. noxia* adults.

Hypothesis:	Slopes equal			Slopes and intercepts equal		
	χ^2	df	P	χ^2	df	P
First series	5.61	4	0.230	22.97	8	0.003
Second series	2.39	4	0.664	12.44	8	0.132

In order to obtain more accurate LC_{50} estimates from these assays, the data from the five replicate assays of each series were pooled to represent two assays, each comprising six doses with *ca.* 100 aphids per dose. These data are presented in Table 8.5.

Table 8.5. Regression analysis of probit mortality and log-dosage data of two bioassays with *B. bassiana* strain GHA against *D. noxia* adults.

Bioassay no.	No. insects	Slope ± SE	LC_{50}[a]	95% FL[a]	χ^2 (df = 4)	G
1	566	1.35 ± 0.09	69	53 – 89	2.31	0.018
2	562	1.28 ± 0.10	97	73 – 125	2.38	0.023

[a] LC_{50} values and their 95% fiducial limits expressed as conidia per square millimeter.

The two probit lines were found to be parallel (χ^2 = 0.29; df = 1; P = 0.588), non-parallelism would have indicated that other factors apart from the stimulus (fungus) affected the response. For final comparison, the POLO program indicated no significant difference between the two probit lines (slopes and intercepts were equal; χ^2 = 4.42; df = 2; P = 0.110; Fig. 8.1).

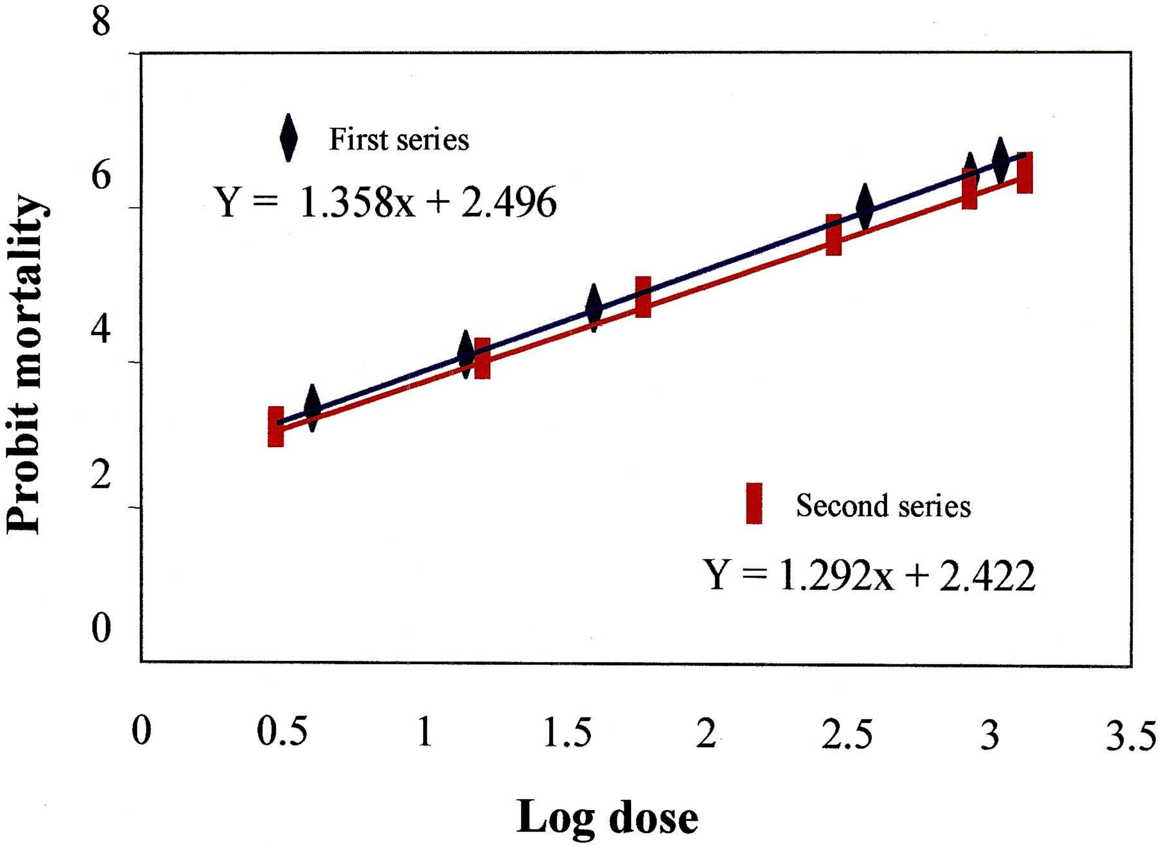


Figure 8.1. Probit lines for two pooled assays

8.4 Discussion

Although significant differences among the five probit regression lines were detected with the first series (P = 0.003), the coefficients of variation (CV) for the slopes were very low, *i.e.*, 19% and 15% in the first and second series, respectively. The fact that no differences were detected among the slopes in either series (P = 0.23 and P = 0.66; Table 8.4), nor among the five probit lines from the second series (P = 0.132), suggests high assay precision. This inference is further supported by the consistently low chi-square values associated with the ten replicate assays and by the finding of no differences between the two series in terms of LC_{50} estimates and slope parameters by standard one-way ANOVA (Table 8.3). Also, the formula used in calculating the fudicial limits incorporates the quantity G, a value that can also be used as a measure of variation (Finney, 1971). In explaining the interpretation of this value Van Ark (1983) states that "*If G is larger than approximately 0.025, the variation of the mortalities is rather large. If G is larger than 0.25, the experimental techniques and/or validity of the probit technique should receive serious attention. If G is equal to or larger than one, the fiducial limits cannot be calculated due to the considerable variation of the points about the probit line.*" The value G for the first and second series (pooled) reported here were below the 0.025 level, once again indicating good bioassay precision (Table 8.5).

This bioassay technique departs significantly from previously published protocols using Hyphomycetes against aphids. One of the most noticeable differences is the low control mortality (< 4%) observed with the technique reported here. Unfortunately, control mortality comparisons with the (cereal) aphid bioassays reported by Feng & Johnson (1990), Feng *et al.* (1990b), Vandenberg (1996), and Vandenberg *et al.* (1998) are not possible as no quantitative details on such mortalities were presented by these authors. However, Mesquita *et al.* (1996) reported control mortality of *ca.* 12% in their assay with *D. noxia* using a Petri dish system with aphids residing on excised leaves. Also, in a study with the brown wheat ear aphid, *Sitobion avenae* (Fitch), Hayden *et al.* (1992) reported control mortality of up to 10%. In other studies with aphids, control mortalities have been considerable, ranging from 8% to as high as 37% (Jackson *et al.*, 1985; Wang & Knudsen, 1993; Miranpuri & Khachatourians, 1995; Chandler, 1992 & 1997).

Notably, the adaptations made to the protocol used in the single-dose assay (see 8.2.3.1 versus Chapter 7) resulted in a significant reduction (24% to 3%) in control mortalities measured in subsequent assays (*i.e.*, multiple dose). According to Goettel & Inglis (1997) control mortalities in pathogen bioassays should, ideally, be kept below 10%. In this regard, the live host-plant system, low RH (40 ± 5%) and reduced aphid handling with this protocol, were considered key aspects in confining control mortalities to levels well below this 10% threshold.

Another important consequence following the above-mentioned adaptations, was the confinement of aphids inside the rearing units. The problem of missing individuals was also experienced by Yeo (2000) who specifically mentioned this aspect as a possible disadvantage in using a live-host-plant system for rearing aphids post inoculation. With the design reported here, success was mainly attributed to the inclusion of the plastic collar effectively sealing the cage onto the pot. Moreover, the importance of including a layer of fine sterile sand is underscored by the fact that, on average, more than 60% of cadavers were found on the soil surface. In this regard, cadavers were easily detectable while the collar allowed for the (daily) removal of dead insects without disturbing the layer of sand on the surface of the soil. Following the single-dose assay, the average number of 'missing' aphids per treatment (assay) was 13 (10.8%), whereas in the control (high aphid numbers) the total number of missing aphids was 19 (*i.e.*, 13.6%). This would have implied an error of *ca.* 10% in mortality calculations given the number of aphids at the start of the assay. Although the numbers of missing aphids were omitted from calculations presented in Table 8.2, it was necessary to address this phenomenon in order to negate the need to count the number of live aphids on the final day of the assay. Obviously, this would save time and effort, allowing a higher throughput of isolates using this assay protocol. The combined average number of missing aphids per treatment and control, recorded during the two multiple-dose assays, was subsequently lowered to only 1.2 (1.2%) and 3 (1.6%), respectively. For practical reasons, this loss (observational) was considered marginal and the associated error acceptable.

The LC_{50} estimate (average of 85 conidia per square millimeter) obtained with this design, was considerably higher than the LC_{50} values of only 0.9 and 2.8 conidia per square millimeter reported by Vandenberg (1996) for two strains of *B. bassiana* against *D. noxia.* In possibly explaining these discrepancies, the three most profound differences between this design and that of Vandenberg (1996) need to be considered:

(1) With this design, aphids moved onto untreated leaves (whole living plants) immediately after spraying where they remained for the duration of the assay. Vandenberg (1996)

sprayed aphids residing on leaves set in water agar where they remained for 20 hours before being transferred (manually) to fresh untreated leaves also set in water agar. Aphid movement during the initial 20 h post inoculation may have resulted in aphids acquiring additional dose simply by moving over the treated leaves (Hall, 1979; Roditakis *et al.*, 2000) and/or agar substrate. As pointed out by Vandenberg (1996), "*Adults may be more likely to move around within an inoculated dish thus exposing themselves to more of the inoculum*".

(2) Vandenberg (1996) again transferred (manually) the aphids to fresh leaves four days later. The two transfers imply physical contact with the aphids, which may have caused injury/stress resulting in higher mortality. Unfortunately, no quantitative indication of control mortality was presented by Vandenberg (1996), other than stating "*Control mortality caused by* B. bassiana *was zero*".

(3) Although Petri dishes were left unsealed, the relative humidity inside dishes ranged from 40 - 70% (Vandenberg, 1996). However, according to that author, a certain level of condensation within the dishes was observed, suggesting periods of humidity closer to 100%. With the design reported here, aphids were maintained inside ventilated cages under greenhouse conditions. There was active air movement due to air-conditioning, and ambient relative humidity never exceeded 45% during the 5-d incubation period post inoculation (*i.e.*, excluding the initial 24-hour incubation period).

Mesquita *et al.* (1996) also reported a relatively low LC_{50} of 17.8 conidia per square millimeter with a strain of the hyphomycete, *Paecilomyces fumosoroseus* (Wize) Brown & Smith, against *D. noxia*. The design by Mesquita *et al.* (1996) was also based on a Petri-dish system with aphids residing on excised leaves. As in the Vandenberg assay, aphids were sprayed while residing on the leaves. In this case, however, the aphids remained on the treated leaves for three days before being transferred to untreated leaves. Again, aphid movement may have resulted in additional inoculum being acquired, leading to a low LC_{50} estimate. With their design, the relative humidity was also high, ranging from 70 – 80%.

Only one report of a bioassay with *B. bassiana* strain GHA against an aphid host has been published. Poprawski *et al.* (1999) reported a LC_{50} estimate of 119 conidia per square millimeter against the brown citrus aphid, *Toxoptera citricida* (Kirkaldy). Their assay design also included the use of a live-host-plant substrate (*i.e.*, seedlings of *Citrus paradisi* Macfad.), but aphids and foliage were sprayed simultaneously. Interestingly, control mortalities reported by Poprawski *et al.* (1999) never exceeded 5%, levels comparable with those reported here. Considering also the very similar environmental conditions used for rearing their aphids post inoculation (*i.e.*, 25 ± 1°C, 50 ± 5% RH and 12 h natural light), these low levels of control mortality underscore the advantage of using a live-host-plant substrate with minimal handling of aphids.

8.5 Conclusion

The aphid bioassay protocol described herein was found to be both precise and practically feasible. Preliminary assays with cereal-aphid species other than *D. noxia* showed good compatibility of the design (Hatting, unpublished observations). The possibility of using this design also for insect hosts other than aphids is a possibility and warrants investigation. In this regard, the use of the design for screening Hyphomycetes against an indigenous leafhopper, *Molopopterus theae* Theron, attacking *Aspalathus linearis* (Brum. f.) Dahlgr. (Leguminosae) in South Africa, is being investigated by ARC-SGI. In addition, this design will facilitate studies on the tritrophic interaction between the wheat host plant (resistant versus susceptible), pathogen, and insect pest.

CHAPTER 9

GENERAL SUMMARY

9.1 Introduction

In contrast to parasitoids and predators, pathogens of aphids have received little attention as natural mortality factors in South Africa. This study, the first of its kind conducted in this country, was aimed at baseline characterization of these antagonists associated with aphids, with special emphasis on the cereal-aphid complex. Furthermore, quantitative data generated during the surveys revealed the comparative level of mycosis as induced by the various fungal species under both dryland (summer- and winter-rainfall regions) and irrigated conditions (summer-rainfall region). At the same time, the incidence of mycoses on the various aphid host species was investigated, suggesting a low level of susceptibility to fungal infection in at least one important cereal aphid species (*i.e., R. padi*).

Prior to these surveys only eight species of fungi had been reported from aphid hosts in South Africa, of which six were Hyphomycetes. Of the nine fungal species recorded from aphid hosts in this study, seven species were recorded from aphid hosts for the first time in this country, bringing to 15 the number of species now associated with the Aphididae (see 9.1.1 below). Moreover, four of the nine species recorded are considered first reports from this country, *i.e.*, the entomophthoraleans *P. neoaphidis*, *C. obscurus*, *C. thromboides*, and *E. planchoniana*.

In addition, seven new aphid-host plant associations are reported, all of which will be included in future editions of the catalogue of the aphids of sub-Saharan Africa (see Millar, 1994) (Mr. Ian Millar, ARC-Plant Protection Research Institute, pers. comm.).

Of the six media tested for *in vitro* isolation of entomophthoralean fungi, Sabouraud dextrose agar supplemented with 1% yeast extract and Sabouraud egg yolk and milk agar proved the most successful and are recommended for future use in this regard. However, isolates of *N. fresenii* and *E. planchoniana* could not be established *in vitro* despite attempts with such sophisticated media as Grace's medium.

9.1.1 List of fungal entomopathogens associated with the Aphididae in South Africa

(Species found associated with cereal aphids under field conditions are printed in bold)

Entomophthorales

Pandora neoaphidis*
Conidiobolus thromboides*
Conidiobolus obscurus*
Entomophthora planchoniana*
Conidiobolus coronatus
Neozygites fresenii

Hyphomycetes

Verticillium lecanii*
Beauveria bassiana*
*Paecilomyces farinosus**
Cladosporium oxysporum
Cladosporium aphidis
Fusarium oxysporum
Cladosporium sp.
Alternaria sp.
Penicillium minioluteum
* aphid-pathogen associations recorded for the first time in South Africa in this study

Findings reported in Chapter 4 were published as follows:

Hatting, J.L., Humber, R.A., Poprawski, T.J. & Miller, R.M. 1999. A survey of fungal pathogens of aphids from South Africa, with special reference to cereal aphids. *Biological Control* 16: 1-12.

9.2 Strategies for exploiting fungal entomopathogens for cereal aphid control in South Africa

Milner (1997) proposed the following four strategies as important when considering the use of entomopathogenic fungi as biological control agents against aphids. In conjunction with these, future research recommendations are appended.

9.2.1 Taking account of epizootics as part of natural control

Under field conditions, aphid pest populations are often challenged by natural epizootics leading to a total or nearly total disappearance of living aphids. In this regard, entomopathogenic fungi are considered beneficial organisms. However, insects reared in the greenhouse are also often attacked by fungal pathogens because conditions such as high humidity, constant optimal temperatures, and artificial light promote disease development. In this regard, pathogens are seen as limiting factors and need to be managed accordingly.

9.2.1.1 Greenhouse control

Epizootics caused by *C. thromboides* in greenhouse rearings of *D. noxia* at the ARC-SGI severely affected several research programmes aimed at developing new resistant wheat cultivars and the introduction of exotic natural enemies. As a result, chemical intervention was considered and six fungicides were tested *in vitro* at a rate of 0.1% active ingredient. The fungicides tested included copper oxychloride (Cupravit), mancozeb + oxadixyl (Sandofan), captab + metalaxyl (Apron C), bitertanol (Baycor), iprodione (Rovral Dip), and mancozeb (Sancozeb). The mean inhibition of 81.1% observed with these fungicides indicates a high level of susceptibility of *C. thromboides* to these chemicals. The dithiocarbamate mancozeb was further evaluated because of strong inhibition (88.0%) of *C. thromboides* and its relatively low cost. Six concentrations were used, ranging from 0.016 – 10.0% active ingredient, enabling one to calculate the fungus growth cut-off point which, in this case, was midway between 1.25 and 2.0%. Subsequently, this fungicide was used at a rate of 1.625% active ingredient per litre of water to treat cages, plants and aphids in greenhouses contaminated with the pathogen. In addition, contaminated greenhouses were fumigated with potassium permanganate ($KMnO_4$) plus formaldehyde (4.3 litres formaldehyde plus 1.8 kg $KMnO_4$ for treatment of 283 cubic metres) while cages were washed with a foaming detergent/sanitizer (Asepto Z) during each aphid cycle. The use of fungicides in combination with other curative and preventative measures proved a successful means in managing this entomophthoralean in rearings of *D. noxia*. Information generated during this study is also considered applicable to other aphid species. Disease-free aphid rearings will be imperative when conducting future laboratory bioassays, a critical component in screening for fungal species/strains with potential for development as mycoinsecticides (see section 9.2.4).

Findings reported in Chapter 3 were published as follows:

Hatting, J.L., Poprawski, T.J. & Miller, R.M. 1999. Managing the entomopathogenic fungus *Conidiobolus thromboides* (Zygomycetes: Entomophthorales) in Russian wheat aphid cultures. *Southwestern Entomologist* 24(2): 99-106.

9.2.1.2 Field control

Under dryland conditions in the summer-rainfall region, infection of *D. noxia* by *P. neoaphidis* reached 50% by mid-November 1996 but occurred too late to protect the wheat crop effectively. However, late-season epizootics could be a factor in ensuring the dissemination of inocula to oversummering populations not only of *D. noxia* but also other cereal aphid species. Under favourable conditions, the development of mycoses at either enzootic or epizootic level could therefore reduce these oversummering populations and possibly the level of infestation of the subsequently planted crop. Surveys conducted in the highlands of Lesotho during March 1999 indicated that *D. noxia* oversummers on several small grains including wheat, barley and oats. Follow-up surveys in Lesotho to quantify the impact of mycoses on *D. noxia* and other cereal aphids are thus warranted. In South Africa, noncereal-aphid hosts appeared to play an important role during the wheat off-season (January through May) in sustaining fungal inoculum of at least three entomophthoralean species, including *P. neoaphidis*. Several grasses in South Africa are known to be suitable alternative hosts for *D. noxia* and future surveys should ideally include such hosts to gain a better understanding of the seasonal impact of entomopathogenic fungi on local populations of this aphid. Moreover, late-season mycosis developing within populations of *D. noxia* feeding on resistant wheat will certainly aid in reducing the overall aphid population and hence the probability for the development of a resistance-breaking biotype.

Under irrigated conditions in the Winterton/Bergville area (summer-rainfall region), *M. dirhodum* was the predominant aphid, with infestation levels ranging from 10.0-98.0%. However, the mean density per tiller was generally low throughout these surveys, reaching only 0.9 and 0.5 aphids per tiller, respectively, during the flowering and milky ripe stages at Bergville during 1997. Respective rates of 13.3% and 20.6% fungal infection were associated with these levels of aphid infestation. A negative correlation found between aphid host density and infection level in > 75% of cases pertaining to this aphid accentuates the role of Entomophthorales-induced epizootics in suppressing the increase of aphid populations (Feng *et al.*, 1991b). For this reason, data generated during this study suggested that at least two entomophthoralean species, *P. neoaphidis* and *C. obscurus*, effectively suppressed the increase of *M. dirhodum* populations on irrigated wheat in this area. Aphids are not considered a problem in this region and farmers are generally not aware of the presence of these insects in their wheat fields.

During the Winterton/Bergville surveys the mode of attachment of cadavers to the substrate, *i.e.*, aphid proboscis versus fungal rhizoids, was found to substantially affect estimates of infection derived from direct cadaver counts. Observations during this study indicated that the level of disease induced by *C. obscurus* (attachment by proboscis; see Chapter 4) was underestimated when direct cadaver counts only were taken into account. This was probably due to the cadavers becoming detached from the substrate (especially under overhead-irrigated conditions), thereby not being recorded as fungus-induced mortalities. In contrast, aphids killed by *P. neoaphidis* were securely attached to the substrate by many rhizoids and remained in that position well into the season. Subsequently, migration of healthy aphids during the late season often led to an overestimation of mycoses expressed as percentages. In conclusion, data generated from direct cadaver counts in a system where two or more pathogens are active may be biased towards those species (*e.g. Pandora* spp. and/or *Entomophthora* spp.) producing rhizoids. This phenomenon should also be taken into consideration when conducting field trials with the aphid-pathogenic Hyphomycetes, which do not produce rhizoids.

All of the five fungal species attacking aphids under field conditions in the winter-rainfall region were recorded from cereal aphids. These species included the three entomophthoraleans *P. neoaphidis, C. thromboides*, and *C. obscurus* and the two Hyphomycetes *V. lecanii* and *B. bassiana.* Once again, *P. neoaphidis* was the predominant fungus, recorded from 98% of aphid cadavers collected. Isolation of *V. lecanii* from an arthropod host is considered a first South African report as previous associations concerned only rust fungi. The largest diversity of cereal-aphid species was recorded during the Langgewens survey. At this site, *Rhopalosiphum padi* was the predominant cereal aphid followed by *D. noxia, S. avenae, M. dirhodum*, and *R. maidis*. However, infection by entomopathogenic fungi occurred only in *D. noxia* (14.3%) and *R. padi* (4.4%). As observed under both dryland and irrigated conditions in the summer-rainfall region, the level of infection of *R. padi* remained below 5% despite a high number of hosts and apparently suitable environmental conditions. These phenomena, observed throughout the study, strongly suggest low susceptibility of *R. padi* to fungal infection. Consequently, the indigenous aphid-pathogenic fungi are not considered important antagonists in the natural regulation of *R. padi*. Inhibitory compounds on the cuticle surface of these aphids are hypothetically proposed as the principal reason for this low susceptibility of *R. padi* to local species/strains of entomopathogenic fungi. In contrast, widespread epizootics together with a large diversity of entomopathogenic fungi (seven species) associated with *D. noxia* in South Africa reflect a high level of susceptibility of this aphid to fungal infection. These observations concur with those reported in the U.S. and Eurasia. Compared with the species diversity of entomopathogenic fungi attacking cereal aphids in irrigated grain crops in the U.S., the three entomophthoralean species *Entomophthora chromaphidis, Zoophthora radicans* (a major aphid pathogen), and *Zoophthora occidentalis* remain unreported from South Africa.

Findings reported in Chapter 5 were published as follows:

Hatting, J.L., Poprawski, T.J. & Miller, R.M. 2000. Prevalences of fungal pathogens and other natural enemies of cereal aphids (Homoptera: Aphididae) in wheat under dryland and irrigated conditions in South Africa. *BioControl* 45: 179-199.

9.2.2 Introduction into a crop to induce an epizootic

Wraight *et al.* (1993) proposed three possible methods for introducing a pathogen into *D. noxia* populations: application of dry-formulated or fresh fungal mycelium or conidia; release of fungus-inoculated hymenopteran parasitoids; or release of fungus-infected aphids. A field study conducted during 1992 at Parma, Idaho, investigated the former two strategies of which the first was considered more successful than the release of fungus-inoculated wasps (Poprawski & Wraight, 1998). Although the hymenopteran wasp was capable of transmitting fungal inocula to aphid hosts (Poprawski *et al.*, 1992b), poor results were obtained during the field trial following the release of inoculated wasps (Poprawski & Wraight, 1998). In this case, the low level of infection (< 1%) was attributed to inadequate duration of leaf wetness and low overnight temperatures. Regarding the first method, successful establishment of *P. neoaphidis* in tillers inoculated with sporulating mycelium did occur, but during more favourable weather conditions. Dissemination of inocula into the surrounding crop was, however, extremely slow with infection rates increasing to only 5.6% nineteen days post inoculation. Similar observations of slow spread of experimentally introduced entomophthoralean fungi also were reported by Milner *et al.* (1982), Wilding *et al.* (1986), and Wilding *et al.* (1990).

Artificial introductions still remain unexplored within an integrated-control system in which resistant host plants occupy the first trophic level. On nonpreferred hosts (*e.g.*, resistant wheat), *D. noxia* populations are generally more broadly distributed and have low population growth rates (Schotzko & Quisenberry, 1998). This would suggest using a vector with superior searching ability in order to locate the host and transmit fungal propagules. In this regard, the recently imported aphelinid wasp (*A. hordei*) (see Chapter 1; Prinsloo, 1998; Prinsloo *et al.*, 2002) could be evaluated as a potential vector of *P. neoaphidis* on resistant wheat in South Africa. However, unstable weather conditions (Poprawski & Wraight, 1998) and low aphid density (Feng *et al.*, 1991a) are considered limiting factors in further developing this strategy (Milner, 1997). From a South African perspective, adverse early-season weather conditions and low aphid populations on resistant wheat accentuate these concerns. Furthermore, only two isolates of *P. neoaphidis* (collected in the winter-rainfall region; Chapter 4) could be established *in vitro*. Attempts with inoculum obtained from several *P. neoaphidis*-infected cadavers collected in the summer-rainfall region failed in yielding cultures. Both isolates grew only on the more complex (and expensive) media tested (*i.e.*, Sabouraud egg yolk and milk agar and Grace's medium); *in vitro* mass production of these isolates could therefore be seen as another constraint in developing this approach. Future surveys aimed at collecting less fastidious strains of *P. neoaphidis* may, however, yield isolates with potential in this regard.

9.2.3 Classical biological control

The successful introduction of a strain of *Z. radicans* into Australia for control of the spotted alfalfa aphid, *Therioaphis trifolii* (Monell) f. *maculata,* was reported by Milner *et al.* (1982). In this case, baseline characterization of the species complex of fungi attacking *T. trifolii* in Australia revealed a very low incidence of disease (Milner *et al.*, 1980). The subsequent importation, release and establishment of a *T. trifolii*-adapted strain of *Z. radicans* from Israel, is to date the only successful classical biological control initiative regarding an aphid pest and a fungal entomopathogen.

Judging from the South African surveys, *Z. radicans* and *E. chromaphidis,* both important pathogens of *D. noxia* elsewhere, probably hold the most potential as candidates for importation to this country. This study revealed that neither of these species or strains thereof seem to attack cereal aphids in South Africa. Similarly, *N. fresenii* has been known to cause widespread epizootics (> 60% mortality) in populations of *D. noxia* in barley and wheat fields in the highlands of Tigrai and Welo, Ethiopia (Poprawski & Wraight, 1998). During this study, however, *N. fresenii* was not recorded from any cereal-aphid host, although *N. fresenii* was observed at epizootic levels in populations of two noncereal aphid species, *Chaitophorus populialbae* and *Hyalopterus pruni.* South African researchers proposed the introduction of *Triplosporium* (= *N.*) *fresenii* for classical control of *D. noxia* (Aalbersberg *et al.*, 1984), although, at the time, these researchers were not aware of the existence of this species in South Africa. However, introduction of an exotic (*e.g.* Ethiopian) strain of *N. fresenii* effective against *D. noxia* should still be considered as a classical biological-control option.

Another approach would be the introduction of cold-hardy strains of *P. neoaphidis* and possibly other species for control of early-season aphid populations requiring disease development under cool, springtime weather conditions. Lesotho has a mean annual temperature of only 10.9°C while most parts of the eastern highlands experience mean annual temperatures below 8°C (Schulze, 1997). In summer, wheat is produced throughout the eastern highlands and surveys in the Qacha's Nek, Sehlabathebe, and Mokhotlong districts are therefore warranted. Follow-up surveys in the cool Naude's Nek Pass region (north Eastern Cape), where *P. neoaphidis* was collected during 1997, should also be conducted.

Rhopalosiphum padi is also seen as a potential candidate for control by classical introduction of a pathogen. However, selection of fungal species/strains effectively targeting this aphid will be a prerequisite. High infection of *R. padi* by *E. planchoniana* (*ca.* 30%) and *P. neoaphidis* (*ca.* 35%) was reported from Denmark (Eilenberg *et al.*, 1996) and Hungary (Basky & Hopper, 2000), respectively. Widespread fungal epizootics within populations of *R. padi* attacking barley under greenhouse conditions at the Njoro Research Station in Kenya were observed during August 1998 (Dr. Peter Arama, pers. comm.). Examination of cadavers collected from this greenhouse epizootic revealed most to be infected by *N. fresenii* (Hatting, unpublished). This Kenyan strain should be considered for importation to South Africa with releases in the winter rainfall region, *i.e.*, Western Cape.

9.2.4 Development of sprayable bioinsecticides

According to Milner (1997) this approach, compared with the three abovementioned strategies, offers most scope for future aphid biological control. Milner (1997) mentioned six ideal characteristics of a biopesticide for aphid control:

(1) inexpensive to mass produce
(2) easy to store
(3) effective over a wide range of temperatures
(4) provide rapid kill at economic doses
(5) wide host range within aphids
(6) minimal non-target effect especially on parasitoids and predators of aphids.

Worldwide, the entomophthoralean *P. neoaphidis* is the most commonly occurring fungal pathogen of aphids (Milner, 1997). Recent attempts to formulate this fungus as alignate granules yielded good results with infection rates of 7 - 27% recorded against live pea aphids, *Acyrthosiphon pisum* (Harris), under laboratory conditions (Shah *et al.*, 1999). However, these authors acknowledged the need for further research on desiccation and storage regimens in order to improve the short-term shelf life of *P. neoaphidis* formulated as alignate granules.

Considering the Hyphomycetes, Milner (1997) mentioned five species of entomopathogenic fungi as worthy pathogens for development as bioinsecticides against aphids. These species include *V. lecanii, B. bassiana, M. anisopliae, P. farinosus*, and *P. fumosoroseus*. During this study, three of these species, *V. lecanii, B. bassiana*, and *P.*

farinosus, were isolated from *D. noxia*, of which *P. farinosus* is considered a first report from this host, worldwide. To date, no attempt has been made locally at developing/formulating any fungus for field evaluation against cereal aphids in this country. Moreover, the use of indigenous biological-control agents, including entomopathogenic fungi, holds several advantages regarding the registration and subsequent commercialisation of such products in South Africa (Krause, 1998).

The Hyphomycetes meet most of the abovementioned requirements regarding development of mycoinsecticides, with at least seven hyphomycete-based mycoinsecticides presently registered worldwide for use against the Homoptera (Shah & Goettel, 1999; Copping, 2001). During 1997, one such product (*i.e.*, Mycotrol® ES) was preliminarily evaluated in South Africa by Hatting & Poprawski (1998) against *D. noxia* feeding on susceptible wheat under dryland field conditions. Although *ca.* 60% control was obtained after the third application directly prior to colonization and subsequent rolling of the flag leaves, subsequent applications did not prevent aphids from increasing (significantly so) on average, from 21 to 45 aphids per tiller. Hatting & Poprawski (1998) concluded that the poor level of control during the critical flag-leaf stage was mainly attributed to the rapid increase in aphid numbers after colonization of the flag leaf (Kriel *et al.*, 1986), and secondly, the cryptic habit of this aphid within rolled flag leaves, possibly shielding the aphids from contact with the fungus.

Subsequent field trials during 1998 and 1999, reported in Chapter 6 of this thesis, were aimed at investigating the efficacy of *B. bassiana* in combination with antibiotic host-plant resistance against *D. noxia*. During both years, an average of *ca.* 61% control was observed following a fungus application during the early flag-leaf stage (GS 39) of the wheat. Interestingly, an earlier spray application (GS 31) resulted in some level of control only during 1998. This phenomenon could be the result of secondary pick-up of fungal inoculum by *D. noxia* due to the high level of aphid activity observed on cultivar 'Limpopo' during that year. This hypothesis of aphids acquiring lethal doses of conidia when moving over treated plant surfaces is further supported by the findings of Hall (1979) and Roditakis *et al.* (2000). The practical implications associated with such a scenario necessitate greater consideration of the actual plant growth stage at which the mycoinsecticide is applied. At the same time, a better understanding of Russian wheat aphid behaviour on the different resistant varieties may assist us in optimising the potential impact of a mycoinsecticide. Research on aphid feeding behaviour using a high-tech feeding monitor (Tjallingii, 1978) is currently underway at ARC-SGI (Me. Vicki Tolmay, ARC-SGI, personal comm.). Moreover, envisaged studies on the tritrophic interaction between host plant, pest and pathogen lead to the development at ARC-SGI of a novel aphid bioassay design employing live host plants for rearing aphids post inoculation (see Chapter 7). In future, this protocol will also be used to investigate aspects 3 – 6 proposed by Milner (1997) (see above).

The precision and general efficacy of this protocol is reflected by the low average CV for slope calculated at only 17%; the low average chi-square of 5.46 ± 2.74 (n = 10 assays); and low control mortality of less than 4%. With this design, an average LC_{50} estimate of *ca.* 85 conidia per square millimeter was calculated for *B. bassiana* strain GHA against adult *D. noxia* (see Chapter 8). This LC_{50} departs significantly from previously published estimates (ranging from 0.9 – 17.8 conidia per square millimeter) for other strains of Hyphomycetes against this aphid. Essentially, the design reported herein largely complies with the six ideal features of a bioassay design mentioned under section 7.1 of Chapter 7. Future research at ARC-SGI will be aimed at rigorous screening of the isolates mentioned in Table 8.1. Also, the two Hyphomycetes *Verticillium lecanii* and *Paecilomyces farinosus* (see Chapter 4) will be assayed using this design.

The information generated with this study is in support of the long-term objective of ARC-SGI in developing an IPM programme against the cereal-aphid complex. Tritrophic interaction studies incorporating host-plant resistance and fungal entomopathogens is seen as a critical component of the IPM strategy being developed by ARC-SGI.

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