EVALUATION OF THE POTENTIAL USE OF ANTAGONISTIC MICROBES ON GRASS SPECIES, TURF AND PASTURE, FOR DISEASE CONTROL AND GROWTH STIMULATION

by

Debra M. Cunningham

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

in the

Discipline of Plant Pathology
School of Applied Environmental Sciences,
University of Natal,
Pietermaritzburg

October, 2003

ABSTRACT

Public tendency, of late, is to reduce liberal use of harmful synthesized chemicals for promoting plant health. Today, biological control is becoming a commonly cited disease control option. Biological control agents (BCAs) not only control disease, but also promote plant growth. Application of biological control is based largely on knowledge of control mechanisms employed by antagonists, as well as the means of application that will ensure that an antagonistic population is established. Knowing the advantages is not the only factor that should be considered before application commences as, the disadvantages must be clearly outlined and explored further before a constructive decision as on implementation of biological control. A literature review was undertaken to provide the necessary technical information about biological control, its potential uses, methods of application, mechanisms of action employed, advantages and disadvantages associated with biological control application, public perceptions and the potential future of biological control.

Diseases encountered within the KwaZulu-Natal Midlands on pasture and turf grasses were determined by a once-off survey conducted over 1999/2000. The aim of the survey was to determine broadly the management practices of farmers and groundsmen in KwaZulu-Natal and the potential impact of these on the occurrence of weeds, insects and diseases. The survey also addressed the level of existing knowledge about biological control and willingness to apply such measures. In the pasture survey, farmers were questioned about: soil type, grass species common used, irrigation, fertilization and liming, grazing programs and weed, insect and disease occurrences and control measures implemented. The same aspects were addressed in a survey to a representative sample of groundsmen (turfgrass production), including also: topdressing, greens base used, drainage systems, mowing practices and decompaction principles. The survey showed correlation between pest incidence and management practices implemented. In terms of pest control, both farmers and groundsmen indicated a stronger preference to the use of herbicides, insecticides and fungicides. Use of fungicides for disease control by farmers is considered an often unfeasible expense, rather more

emphasis was placed on implementing cultural control methods. At present farmers do not apply biological control strategies, but they did indicate much interest in the topic. Alternatives to current, or lack of current, disease management strategies are important considerations, with two new diseases identified in the KwaZulu-Natal Midlands just within the period of this thesis. Biological control strategies are implemented by 8% of the groundsmen surveyed, with emphasis being placed on augmenting the already present natural predators rather than the introduction of microbial antagonists.

Although often mis-diagnosed by farmers Helminthosporium leaf spot is a common disease in the KwaZulu-Natal Midlands on *Pennisetum clandestinum* (kikuyu). This disease reduces pasture quality and detracts from the aesthetic appearance and wearability of turfgrasses. Helminthosporium leaf spot is incited by a complex of causal agents, *Bipolaris* was confirmed as the casual agent of Helminthosporium leaf spot on kikuyu at Cedara. Disease control by two BCAs, *Bacillus* (*B. subtilis* Ehrenberg & Cohn.) and *Trichoderma* (*T. harzianum* Rifai), as commercial formulations was tested against the fungicide, PUNCH EXTRA®. *In vitro*, *Trichoderma* was shown to be aggressive in controlling *Bipolaris* sp. *In vivo,* disease control achieved with *Trichoderma* kd was comparative with PUNCH XTRA® but not statistically different ($P \geq 0.05$). *Trichoderma* and *Bacillus* provided better disease control in comparison to an untreated control.

Improved growth of *Lolium* sp. was determined *in vitro*, with *Trichoderma* kd and *Bacillus* B69 treatments. The microbe-based treatments accounted for growth stimulation, with significant ($P \leq 0.05$) growth differences noted. A microbial activator, MICROBOOST® was added to the treatments to improve microbial efficiency. Improved plant growth with MICROBOOST® applications was shown.

Improved growth associated with microbial treatments, *Trichoderma harzianum* kd; *Bacillus subtilis* B69 and *Gliocladium virens* Miller, Gibens, Foster and con Arx. ,was also determined *in vivo* at Cedara, on *L. perenne* L., *Festuca rubra* L. and *Agrostis stolonifera* L. Establishment of a suppressive soil with antagonistic microbes resulted in significant ($P \leq 0.05$) effects on final grass coverage (except *G. virens*), as well increased root and

shoot lengths ($P \leq 0.05$). Increased germination rates, as expressed *in vitro,* were not shown *in vivo.* Microbial activity with the application of MICROBOOST® showed little effect on germination but increased root and shoot lengths significantly ($P \leq 0.05$). Increased weed growth associated with the treatments (except *G. virens*) was considered a drawback of the microbial-treatments.

Microbial treatments were also applied to pasture grasses. An *in vitro* grazing trial was established at Cedara, using *L. multiflorum* L. to evaluate the microbe-based treatments *Trichoderma* kd, *Bacillus* B69 and *G. virens* for improved pasture establishment and for increased grazing preference by Dohne Merino sheep. *Trichoderma* kd was associated with increased dry and wet biomass, but lower dry matter yields in comparison to the control. Only *G. virens* accounted for a higher dry matter percentage than the control. However, differences between the control and the microbial treatments was very small and not significant ($P \geq 0.05$). Of the three grazing observations made, sheep showed no grazing preference to plots with or without microbial treatments

In general, the body of this research has shown that microbial treatments have the potential for increased disease control and growth stimulation of grasses. However, lack of significant differences between microbial treatments and controls has raised the question as to effect of external factors on microbial activity and survival, especially *in vivo.* This raises the question as to the validity of the use of microbial treatments where growth conditions cannot be controlled, remembering that the cost of establishment must be covered by the economic returns from utilization.

DECLARATION

The *in vitro* experimental work presented in this thesis was conducted in the Crop Protection Disease Clinic Laboratory, Cedara: Department of Agriculture and Environmental Affairs, KwaZulu-Natal and at the University of Natal, Pietermaritzburg, KwaZulu-Natal. *In vivo* trial work was conducted at Cedara, Department of Agriculture and Environmental Affairs, KwaZulu-Natal. This thesis was completed part-time from 1999-2003 under the supervision of Professor Mark D. Laing and Dr. Patricia M. Caldwell of the University of Natal.

Research reported in this thesis, has not been submitted in any other form to another University for degree purposes. Unless otherwise indicated, all research is from my own investigations.

Debra M. Cunningham

October 2003

ACKNOWLEDGMENTS

To the following I extend acknowledgment:

Professors M.D. Laing and Dr. P.M. Caldwell, for their patience in helping me to complete this thesis and for their guidance, constructive criticisms and sound advice during the course of the investigations. Special thanks to Dr. P.M. Caldwell for reviewing and editing the manuscript before submission and for her encouragement along the journey to completion.

Crop Protection and Pasture Science at Cedara, Department of Agriculture and Environmental Affairs for their assistance in completing *in vitro* and *in vivo* trial work. Providing laboratory space, equipment and technical assistance, I thank Eve du Preez and Laura Miles at the Crop Protection Disease Clinic. For assistance in obtaining weather data, I thank Neil van Rij at Crop Protection. Providing land, labour, pasture grass seed and technical assistance, I thank John Cunningham at Pasture Science.

Biometry Section Cedara, Department of Agriculture and Environmental Affairs for statistical analysis of data collected. For their endless patience and explanation of statistical analyses, I thank Cathy Stevens and Margie Whitwell.

The Centre for Electron Microscopy, University of Natal Pietermaritzburg for technical assistance in the use of the Scanning Electron Microscope.

Mayford Seeds, I thank Sue Allan for supplying the turfgrass seeds. Microbial Solutions and Plant Health Products cc, I thank for supplying the microbial formulations.

Lastly, special mention to my parents John and Caroline Cunningham for their sacrifices and support. To my husband, Andrew Whitley, for always believing I could do it and for his help and understanding during my years of studying.

To my Father, John Cunningham

CONTENTS

CHAPTER 4

POTENTIAL FOR THE BIOLOGICAL CONTROL OF *HELMINTHOSPORIUM* LEAF SPOT ON THE PANICOID GRASS USING AMENDED BIOLOGICAL CONTROL AGENTS

LIST OF ACRONYMS

ADF	Acid detergent fibre
ANOVA	Analysis of variance
AUDPC	Area under the disease progress curve
BCA(s)	Biological control agent(s)
Bt	*Bacillus thurengensis*
Ca	Calcium
Cu	Copper
CV	Co-efficient of variation
DAP	Days after planting
d.f	Degrees of freedom
DM%	Dry matter percentage
DW	Dry weight
EU	European Union
FD%	Final disease percentage
IPM	Integrated Pest Management
K	Potassium
LAN	Limestone ammonium nitrate
LSD	Least significant difference
Mg	Magnesium
Mn	Manganese
N	Nitrogen
Na	Sodium
NDF	Neutral detergent fibre
NH_4SO_3	Ammonium sulphate
NPN	Non-protein content
NSC	Non structural carbohydrates
NZ	New Zealand
P	Phosphorus
REML	Restricted maximum likelihood
R_2	Regression value
S.A.	South Africa
SEM	Scanning Electron Microscopy
U.S.	United States
WAP	Weeks after planting
WW	Wet weight
X^2	Chi-square
Zn	Zinc

INTRODUCTION

A grassland, be it for grazing or amenity purposes, if established and maintained correctly will provide a sustainable economic return. Establishment of a cultivated grassland is an expensive endeavour (Whitehead and Dunn, 1991), and establishment rates are often affected by a number of abiotic and biotic factors (Carter, 1987; Bartholomew, 2000). Poor establishment is undesirable as the grass is unable to "recuperate" from grazing or intensive use. The plants are also less able to resist pathogen attack, increasing production costs in terms of fungicide use. Severe weed infestations are also a result of poor grass establishment, and production costs are once again increased due to herbicide use. In severe cases of poor establishment (due to weed or disease outbreaks) there is the additional cost of having to re-establishment the area. Fear of poor pasture establishment is considered to be a contributing factor as to why farmers are discouraged from exploring the use of new cultivars or farming practices (Wheeler, 1987).

The aim of this thesis was to determine the potential effect of biological control agents (BCAs): *Trichoderma harzianum* Rifai, *Bacillus subtilis* Ehrenberg & Cohn. and *Gliocladium virens* Miller, Gibens, Foster and con Arx., for disease control and growth stimulation of pasture and turf grasses. The concept of biological control is derived from suppressive soils (Agrios, 1997). Suppressive soils are defined as soils where antagonists become established with little or no disease; or where disease occurs at first, declining as the antagonists replace the pathogens (Cook, 1988). The antagonistic microbes listed for potential biological control were initially derived from suppressive soils and cultured into commercial products for potentially increasing plant health. Amendment of BCAs to turf grass soils is a reality today. Application of BCAs not only offer disease control, but also stimulate growth (Tainton and Klug, 2002). Where antagonistic microbes offer effective disease control, biological control can be considered an alternative to fungicide applications. A number of BCAs have been shown to be compatible with fungicide applications, providing an integrated approach to disease control (Papavizas, 1985). Within specific niches, the application of BCAs "fits" better than fungicide

applications (Harman, 2000). One of these niches is organic farming. Public perception about BCAs and their potential use was determined by means of a once-off survey of pasture and turf managers in KwaZulu-Natal (1999/2000).

Pasture production literature in South Africa generally neglects to outline pasture diseases commonly encountered and their potential effects on pasture production. A once-off survey revealed that pasture farmers encounter a number of diseases. Where pasture diseases are mentioned in the literature, information is often lacking detail. Disease control options are often limited to the removal of infected foliage either by means of grazing or mowing, improving fertilization and increasing management (du Plessis, 1991). These methods have the potential to amplify disease symptoms on new growth rather than suppress their expression, based on poor management practices. Fungicide applications are considered only economical for hay and seed production (Johnstone and Barbetti, 1987). The impracticality of applying fungicides to pastures lies in the withholding periods associated with the chemicals and grazing potential. In terms of commonly encountered turf diseases, information and disease control options are more prolific, but is limited to the American scenario (Smiley *et al.*, 1992; Couch, 1995). Disease control lists options ranging from the use of various fungicides to simple cultural practices. The use of chemicals on amenity grasses raised many questions as to human safety upon exposure to such chemicals. Fungicide applications to grasses are also considered ineffective against soil and root diseases (Tainton and Klug, 2002). Application of BCAs poses an safe alternative for disease control, for both soilborne (Lo *et al.*, 1996; Kim *et al.*, 1997) and foliar diseases (Lo *et al.*, 1997; Harman, 2000).

A commonly encountered disease on *Pennisetum clandestinum* (kikuyu) by KwaZulu-Natal farmers and turf managers, as well as observed on many home lawns, is kikuyu leaf spot referred to in this thesis, as Helminthosporium leaf spot. Disease symptoms are associated with the leaf blades, where reddish-brown to purplish-black lesions appear. As lesions expand and girdle the leaf, chlorosis and die-back occurs. In severe cases crowns, stems and rhizomes are also infected (Couch, 1995). *Bipolaris* sp. also occur on other warm- and cool-season grasses. Kikuyu is the most commonly used grass species

for the establishment of summer pastures, producing reasonable winter foggage (Bartholomew, 1991), while for turf production kikuyu is used for establishment of cricket outfields, winter sportfields (Tainton and Klug, 2002) and golf fairways. Disease symptoms intensify with increased utilization of turf. Control methods include cultural practices that induce optimum growing conditions, and fungicide applications (Tainton and Klug, 2002). In pasture and turf production cultural practices such as monoculture production, mowing and grazing may result in an increase in disease occurrence.

The use of amended BCAs for control of Helmithosporium leaf spot was considered as an alternative to fungicide use. Disease control may be due to systemic acquired resistance upon colonization of the roots (Harman, 2000). A reduction in disease symptoms on new growth may also be due to reduced disease inoculum in the thatch layer (Cross, 1982). Use of BCAs often results in increased plant vigour and growth (Harman, 2000; Anon, 2002b). In grasses, improved vigour results in quicker plant recovery, improving grazing management and pasture productivity. In turf grasses, stimulated growth results in improved wear and recovery, reducing patchy turf. Increased growth is also associated with greater thatch, which for rugby fields for example, creates more "cushioning". Stimulated growth has also shown to be associated with improved chlorophyll concentrations and colour (Raviv *et al.,* 1998). The microbe based product, BIOSTART®[1] for example, attributes this increased growth activity to increased nitrogen and phosphorus availability, as well as plant hormone production (Anon, 2002b). The mechanisms employed by the microbes are, however, varied and often unknown.

For successful adoption of BCAs, it is essential that amended microbes have no detrimental effects on the ecosystem into which they are amended. Potential pathogenic effects of the antagonistic microbes on humans, grazers and the grass plants, must be determined before extensive application. These factors are addressed in terms of the registration requirements (Thomas and Willis, 1998). The application of antagonistic microbes may simply serve to re-establish a natural balance, colonizing the rhizosphere

[1] Microbial Solutions (Pty)Ltd., P.O. Box 103, Kya Sand, 2163, South Africa. Tel: (+27) 11 462 2408

by displacing other micro-organisms (Harman, 2000). This raises the question as to the amendment of cultured antagonists into a soil ecosystem or simply to augment the antagonists already present (Thomas and Willis, 1998).

The future of biological control lies not only in the visual effects of BCAs on plant vigour and health, as shown in this research, but also understanding rhizosphere activities in terms of the interactions between the pathogen and antagonistic microorganisms. Biological control must offer safe, economic and environmentally sustainable effects on plant growth (Harman, 2000). If this is achieved, then the future for BCAs is promising.

REFERENCES

Agrios, G. 1997. Plant Pathology, 4th edition. Academic Press, California: United States of America.

Anon, 2002a. Agro-organics: products list. http://www.agro-organics.co.za (accessed 24 July 2003).

Anon, 2002b. Microbial solutions: product range. http://www.microbial.co.za (accessed 24 July 2003).

Bartholomew, P.E. 1991. Adaption of pasture species. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Bartholomew, P.E. 2000. Establishment of pastures in humid regions. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 156-171.

Carter, E.D. 1987. Establishment and natural regeneration of annual pastures. In: J.L. Wheeler, C.J. Pearsons and G.E. Robards (eds). Temperate pastures: their production, use and management. CSIRO, Collingwood:Australia. p. 35;41.

Cook, R.J. 1988. Management of the environment for the control of pathogens. In: R.K.S.Wood, F.R.S. Way and M.J. Way (eds). Biological control of pests, pathogens and weeds: developments and prospects. Philosophical Transactions of the Royal Society of London, Volume 318:1189. Royal Society, London: United Kingdom. p. 173.

Couch, H.B. 1995. Diseases of turfgrasses. 3rd edition. Krieger Publishing, Florida: United States of America.

Cross, G.W. 1982. The management of turfgrass. In: G.M. Brockett (ed). Grass for turf and revegetation. Grassland Research, Cedara, Pietermaritzburg: South Africa. p. 25-35.

du Plessis, T.M. 1991. Problems on pastures: pitfalls and pointers. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Harman, G.E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease **84:** 377-393.

Johnstone, G.R. and M.J. Barbetti. 1987. Impact of fungal and virus diseases on pasture. In: J.L. Wheeler, C.J. Pearsons and G.E. Robards (eds). Temperate pastures: their production, use and management. CSIRO, Collingwood: Australia. p. 235-248.

Kim, D., R.J. Cook and D.M. Weller. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology **87:** 551-558.

Leben, C. 1985. Introductory remarks: biological control strategies in the phylloplane. In: C.E. Windels and S.E. Lindow (eds). Biological control on the phylloplane. American Phytopathological Society, Minnesota: United States of America. p. 1-5.

Lo, C.T., E.B. Nelson and G.E. Harman. 1996. Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum.* Plant Disease **80:** 736-741.

Lo, C.T., E.B. Nelson and G.E. Harman. 1997. Improved biocontrol efficacy of *Trichoderma harzianum* 1295-22 for foliar phases of turf diseases by use of spray applications. Plant Disease **81:** 1132-1138.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Annual Review of Phytopathology **23:** 23-54.

Raviv, M., B.Z. Zaidman and Y. Kapulnik. 1998. The use of compost as a peat substitute for organic vegetable transplant production. Compost Science and Utilization **6:** 46-52.

Smiley, R.W., P.H. Dernoeden and B.B Clarke. 1992. Compendium of turfgrass diseases, 2nd edition. American Phytopathological Society, Minnesota: United States of America. p. 34-36.

Tainton, N.M. and J. Klug. 2002. The cricket pitch and its outfield. University of Natal Press, Pietermaritzburg: South Africa. p. 99, 122-123.

Thomas, M.B. and A.J. Willis. 1998. Biocontrol - risky but necessary? TREE **13:** 325-329.

Wheeler, J.L. 1987. Pastures and pasture research in southern Australia. In: J.L. Wheeler, C.J. Pearsons and G.E. Robards (eds). Temperate pastures: their production, use and management. CSIRO, Collingwood: Australia. p. 4-5.

Whitehead, E.N.C. and I.S. Dunn. 1991. Economics of pastures. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

CHAPTER 1

AN OVERVIEW OF BIOLOGICAL CONTROL

1.1 INTRODUCTION

The use of microorganisms in the production of pharmaceutical antibiotics for the control of human aliments is common practice (Marrone, 1999). Could therefore, this exploitation not be successfully applied to the biological control of plant diseases too?

Biological control is defined as a means by which natural enemies are manipulated for the reduction and suppression of pest populations (Orr and Baker, 1997a). As a science biological control was implemented in the19th century (Waage and Greathead, 1998). Today, biological control is viewed as a natural phenomenon with high potential for disease management (Cook, 1990; Thomas and Willis, 1998). Although still relatively small, the commercialization of biological control agents (BCAs) is today a reality (Agrios, 1997; Koch, 1999). Growing interest in BCAs and their commercialization is largely attributed to increased public concern over the environmental impact of synthetic pesticides and potential health hazards associated with their use. A more rational approach to disease control, i.e., integrated pest management (IPM), has also contributed to the growing interest in biological control.

This literature review serves as an introduction to the concept of biological control and plant growth stimulatory effects of antagonistic strains of the microbes *Bacillus, Trichoderma* and *Gliocladium*. The potential of these antagonists have been studied in Chapters 4 to 6 that follow.

1.2 THE RHIZOSPHERE AND SUPPRESSIVE SOILS

The concept of biological control is derived from suppressive soils (Agrios, 1997). Therefore, an understanding of soil complexities and the interactions and relationships that occur within the rhizosphere, are advantageous to the development of biological control. Suppressive soils are therefore defined as soils where antagonists become established with little or no disease; or where disease occurs at first, declining as the antagonists replace the pathogens (Cook, 1988). These soils play an important role in the renewed interest shown in alternatives to synthetic chemical control. However, the establishment of a suppressive soil is slow and microbe manipulation is difficult and often impractical (Kerry, 1992). Successful colonization determines the degree of antagonistic potential expressed by the beneficial microbes.

Colonization is further determined by the carrying capacity that the rhizosphere can support (Handelsman and Stabb, 1996). The rhizosphere is the site on the root surface of intense activity between plant pathogens and antagonists (Schroth and Becker, 1990). In terms of biological control, research emphasis is based on the numerous interactions that occur within the rhizosphere, and the direct and indirect effects of these interactions on plant performance (Kapulnik, 1996). These interactions contribute to the physical, chemical and biological complexities of the soil, determining susceptibility or resistance to disease (Singh and Faull, 1988; Bazin *et al.*, 1990; Dandurand and Knudsen, 1993).

1.3 BIOLOGICAL CONTROL AGENTS

Numerous fungal and bacterial strains have been found to be antagonistic. The potential of *Bacillus subtilis* Ehrenberg & Cohn., *Trichoderma harzianum* Rifai and *Gliocladium virens* J.H. Miller, J.E. Gidens, A.A. Foster & von Arx, as BCAs will be addressed in this chapter. In the United States of America, *Trichoderma* and *Gliocladium* spp. are widely used commercial fungal BCAs, whereas *Bacillus subtilis* is the most widely used bacterial BCA.

1.3.1 DISEASE CONTROL INDUCED BY BCAS

Biological control of pathogens, is defined as the total and partial destruction of pathogen populations by other organisms (Agrios, 1997). There are a large number of microorganisms which exist in a biological balance within plant tissues or within the soil, disease occurrence being attributed to induced disturbances of this balance (Schippers, 1992; Tjomas, 1992). Complex microbial interactions and the wide range of environmental conditions that exist, make it unlikely that any one antagonistic strain is responsible for the suppression of a single disease (Handelsman and Stabb, 1996). The concept of using a combination of BCAs, to offer a wider spectrum of control, has been considered (Dandurand and Knudsen, 1993; Guetsky *et al.*, 2001). Future research programs have been assigned to determine the compatibility of BCAs in their activities.

1.3.1.1 *Bacillus subtilis* as a BCA

Bacillus spp. as BCAs of seed- and soilborne diseases have been studied extensively and there are numerous publications on the efficacy of *Bacillus* spp., especially inoculations of *B. subtilis* (Marrone, 1999). Some strains have proved more effective than fungicides (Baker *et al.*, 1985; Tronsmo, 1992). With misuse of fungicides resulting in increased pathogen resistance, applications of *B. subtilis* as an alternative treatment is advantageous (Berger *et al.*, 1996). *Bacillus subtilis* has proved effective against foliage, soilborne and postharvest diseases, specifically those caused by *Phytophthora*, *Pythium* and *Rhizoctonia* species. However, *B. subtilis* when used to control postharvest diseases, may become pathogenic (Hazen, 1989). In terms of pasture and turf grass diseases, the benefits of *Bacillus* are being realized. In the United States of America (U.S.A.), strains of *B. subtilis* are available as commercial formulations for the control of damping-off and brown patch (*Rhizoctonia*)(Kim *et al.*, 1997).

Bacteria occur naturally within the environment and are considered to be an "environmentally friendly" mechanism of disease control. Being an endospore former, *Bacillus* spp. are able to survive long-term within the soil environment, as well as display a long and stable shelf-life as a commercial formulation (Handelsman and Stabb, 1996; Nebec, 1997). The potential of BCAs comprising *Bacillus* sp. is therefore great. These BCAs are applied directly to the soil or as a seed treatment prior to planting. Soils where

antagonistic *Bacillus* sp. have been identified may simply require management of the soil environment, to promote further colonization of the indigenous antagonists.

1.3.1.2 *Trichoderma harzianum* and *Gliocladium* spp. as BCAs

Mycoparasites too, have the potential for biological control. Although the fungi are considered predominately antagonistic, *Trichoderma* spp. are known to incite disease in isolated cases (Samuels, 1996). *Trichoderma* and *Gliocladium* strains are effective BCAs against several fungal soil pathogens, providing an alternative to fungicides (Harman, 2000). Diseases controlled by these fungi include: *Pythium* (Lo *et al.*, 1996; Lo *et al.*, 1997; Koch, 1999), *Phytophthora* (Chambers and Scott, 1995), *Sclerotium* (Knudsen *et al.*, 1991; Abd-El-Moity, 1992; Lo *et al.*, 1996; Lo *et al.*, 1997), *Verticillium* (Agrios, 1997), *Fusarium* (Ahmad *et al.*, 1995; Agrios, 1997) and *Rhizoctinia* (Abd-El-Moity, 1992; Lo *et al.*, 1996; Lo *et al.*, 1997; Koch, 1999). Fungi and yeasts, responsible for pre- and post-harvest diseases, may also be controlled (Deacon and Berry, 1992; Agrios, 1997; Lo *et al.*, 1997; Harman, 2000). Release of new biological products is growing rapidly. In terms of turf disease, *Trichoderma*-based biological control products exist for brown patch; dollar spot (*Sclerotinia*) and Pythium root rot and blight. Applications effectively reduce disease symptoms, enhancing turf quality (Lo *et al.*, 1996; Lo *et al.*, 1997).

The potential integration of mycoparasites and fungicides for disease control exists as there are fungicide resistant biotypes (Yuen *et al.*, 1994; York, 1996; Harman, 2001). For example, non-target effects of fungicides on *T. harzianum* have been reported (Papavizas, 1985; Abd-El-Moity, 1992). Genome modification of *Trichoderma* and *Gliocladium* spp. to produce fungicide-resistant biotypes is also successful, as is mutagenesis by UV radiation (Papavizas, 1985).

1.3.2 PLANT GROWTH INDUCED BY BCAS

Commercial application of mycoparasites offers protection against root-diseases and may enhance plant growth (Kim-Jeong *et al.*, 1992; Kapulnik, 1996; Nebec, 1997; Harman, 2000). Numerous antagonistic microbes colonize the rhizosphere, replacing the existing microflora, forging a close relationship with the plant root (Harman, 2000).

1.3.2.1 Examples of plant growth promoting BCAs

Trichoderma spp. are the most commonly documented growth promoters. *Trichoderma harzianum* is known to increase germination percentages, seedling emergence and establishment (Raviv *et al.,* 1998), plant height, leaf area, dry weights of shoots, stems and roots (Windham *et al.*, 1986; Baker, 1992; Kleifeld and Chet, 1992; Ousley *et al.*, 1993; Harman, 2000), chlorophyll concentrations (Raviv *et al.*, 1998), flowering and blooms per plant (Chang *et al.*, 1986; Ousley *et al.*, 1994). A further advantage associated with *T. harzianum* use, especially in seedling production, is the carry over of the BCA into the rhizosphere of the transplanted seedlings, promoting re-establishment (Chang *et al.*, 1986; Kim-Jeong *et al.,* 1992; Harman, 2000).

Plant growth stimulation may not be expressed immediately in all scenarios (Kim-Jeong *et al.*, 1992; Ousley *et al.*, 1993). This could be attributed to poor fungal growth. Factors affecting fungal growth are the microbial strain, culture age, application rate, colonization rate, chlamydospore production and the media's fungistatic nature (Papavizas, 1985; Ousley *et al.*, 1993; Lewis *et al.*, 1998). Hyphal growth and expansion into the soil may also require chemical stimulation (Knudsen *et al.,* 1991). Soil pH also effects fungistasis (Papavizas, 1985).

Plant-growth-promoting-rhizobacteria increase plant growth via a variety of mechanisms, including the production of plant growth regulation phytohormones, suppression of root pathogens (Cook, 1990; Schroth and Becker, 1990) and increased absorption potentials through increased root growth. Nitrogen fixation by antagonistic *Bacillus* spp. has also been proposed (Curl and Truelove, 1986). Often antagonists require mutation to enhance plant growth stimulation and biological control potentials of the wild types. Mutants must survive and compete on the rhizosphere (Fiddaman and Rossall, 1995).

1.4 APPLICATION OF MICROBIAL BCAs

A delivery system that is practical and economic is important for BCA development (Lewis *et al.*, 1998; Rodham *et al.*, 1999).

1.4.1 BCA APPLICATION

Prior to BCA application it is vital that the biology of the antagonistic microorganism is understood (Orr and Baker, 1997a). This will affect the application rate, inoculum activity at application and application timing (Schroth and Becker, 1990; Fravel, 1992). Mass inoculation could be effective in suppressing pathogen activity, but inoculum quality must also be considered (Curl and Truelove, 1986; Jutsum, 1988; Orr and Baker, 1997b). However, mass inoculation can be counter-productive because once the carrying capacity is exceeded, the microbes die (Handelsman and Stabb, 1996). It has been suggested that these dead or dying BCAs provide a nutrient source for the proliferation of soil pathogens (Dandurand and Knudsen, 1993). Many BCAs require organic or inert carriers to provide an establishment substrate for immediate colonization after inoculation (Papavizas, 1985; Dandurand and Knudsen, 1993; Lewis *et al.*, 1998). Chemical or heat pre-treatments of the growing medium, before inoculation, will create a biological vacuum favouring the establishment of amended antagonistic populations (Papavizas, 1985).

Delivery methods of BCAs are numerous and include: root dips (Kerr, 1980), seed treatments (Kleifeld and Chet, 1992; Fiddaman and Rossall, 1995; Cliquet and Scheffer, 1997), conidial suspension (Kleifeld and Chet, 1992; Lo *et al.*, 1997), dusting (Papavizas, 1985), seed pelleting (Lutchmeah and Cooke, 1985; Knudsen *et al.*, 1991), fluid-drilling gels (Conway, 1986), granules (Dandurand and Knudsen, 1993; Lo *et al.*, 1997; Koch, 1999), wettable powders (Koch, 1999) and seed primers (Harman and Taylor, 1988; Callan *et al,* 1990). BCAs are also delivered by trickle-drip irrigation (Rahe, 1988), liquid drench, bulk organic matter, fermenter biomass (Ousley *et al.*, 1993; Ousley *et al.*, 1994; Lewis *et al*, 1998) or polymer prills. Delivery has even been attributed to conidia adhering to insects (Peng *et al.*, 1992). Common application methods include a direct application to plant-propagative material or the amendment of seedling trays/beds before or

immediately after planting (Chet and Baker, 1980; Curl and Truelove, 1986; Kleifeld and Chet, 1992; Ousley *et al.*, 1994; Nebec, 1997). Biological control agents should be regarded as preventative measures of disease control, forming part of a total management approach (Kerry, 1992; Harman, 2000). This includes control measures such as improved cultural practices (Nigam and Mukerji, 1988; Tu, 1992), soil solarization (Cook, 1990; Fravel, 1992; Fiddaman and Rossall, 1995; Koch, 1999) and agrochemical use (Agrios, 1997).

For the sake of public health and the environment, BCAs must be thoroughly tested before release. Here any effects on the environment, public health or non-target organisms must be identified (Schroth, 1992; Ousley *et al.,* 1994; Thomas and Willis, 1998; Koch, 1999). Professional standards associated with synthetic chemicals should pertain to microbial products too (Orr and Baker, 1997a, 1997b). Commercialization and successful application of BCAs is further influenced by storage viability. *Bacillus* spp. form endospores, allowing for vacuum packed storage at high temperatures (40°C) and a shelf life greater than 6 months (Rodham *et al.*, 1999). Mycoparasite viability is dependent on storage conditions and an appropriate storage medium (Cliquet and Scheffer, 1997; Prakash *et al.*, 1999). Temperature is another important environmental factor. For example, *T. harzianum* conidia viability does not exceed 4-6 months at 15°C when stored on a solid substrate (Cliquet and Scheffer, 1997). The acceptable shelf-life in this market, however, requires stability for a period of one year (Nebec, 1997, Rodham *et al.*, 1999).

1.4.2 INTEGRATED PEST MANAGEMENT (IPM)

Much emphasis has been placed on integrating biological control and host resistance, with limited use of synthetic chemicals. Implementation of this, however, is dependent on maintaining high yields and good quality produce (Thomas and Waage, 1996).

Integrated pest management has numerous advantages, such as better overall management (Hartman, 1996), economic benefits, pest management with the lowest environmental impact, reduced use of synthetic chemicals and the use of more target specific and less toxic chemicals (Orr and Baker, 1997a). In turfgrass management

alone, cultural techniques such as coring, thatch control, drainage, correct mowing heights, carefully scheduled irrigation and the use of fungicides to control disease outbreaks have been practised for years by groundsmen (Grant, 1993; Thomas and Waage, 1996). In the U.S.A., IPM is gaining popularity, especially as the government has initiated programmes to assist producers in reducing synthetic pesticide use (Lo, 1998; Marrone, 1999). In South Africa (S.A.), insect and disease management is still reliant on chemical use, with IPM considered to be in the initial stages of implementation.

A major disadvantage facing IPM is the lack of an understanding of natural enemies (antagonists) and reliance on synthetic chemicals that are more than often assocaited with stable disease control. A review by Williamson (1998), attributes the lack of IPM support to poor communication channels between farmers, researchers and agricultural advisors. Informal education and innovative programmes aimed directly at farmers will increase their understanding and application of IPM and biological control. A further disadvantage of IPM is that it requires more intensive management. Although IPM is considered at present to be time consuming, the advantages will be realized in the future especially in terms of sustainable farming (Lo, 1998; De Ceuster and Hoitink, 1999).

1.5 MECHANISMS OF ACTION IMPLEMENTED BY BCAs

Defence mechanisms that exist between antagonists and pathogens must be realised and exploited for improvement and wider application of BCAs (Fravel, 1988; Lo, 1998). Disease control and/or growth stimulation employed or induced by microbial agents is often linked to a plant's natural control mechanisms. Disease control and growth stimulation induced by microbial agents are inter-dependent because by controlling potential pathogens, growth is stimulated instead of suppressed (Windham *et al.*, 1986). Various mechanisms of disease control and growth stimulation have been proposed, however, some of the proposed mechanisms are only speculative (Curl and Truelove, 1986; Mandeel and Baker, 1991; Lynch, 1992).

1.5.1 PROPOSED MECHANISMS EMPLOYED BY BCAS FOR DISEASE SUPPRESSION

Some proposed mechanisms are accepted based on conclusive evidence, while others still require verification (Fravel, 1988). Proposed mechanisms of disease control cannot be viewed as separate entities, often collaboration between mechanisms is needed to achieve the desired effect (Lo, 1998).

Documented proposed mechanisms are as follows:

Competition between the pathogen and antagonist populations for nutrients and space on the root surface is high (Lo, 1998). Biological control agents do, however, display a greater ability to utilize nutrients, depriving pathogen populations (Fravel, 1988; Handelsman and Stabb, 1996). Many pathogens are also stimulated by plant exudates. Competition for these exudates between the pathogen and antagonist is also high in the soil rhizosphere, often resulting in the antagonist outcompeting the pathogen (Lo, 1998).

Inducing or enhancing a plant's natural defence system (induced resistance) to reduce infection by a pathogen (Fravel, 1992; Handelsman and Stabb, 1996; Krebs *et al.*, 1998). The inoculation of potting media with commercial sources of *B. subtilis* and *T. harzianum* results in disease control. The antagonists are transferred with transplanting (Nebec, 1997). *Trichoderma harzianum* also has the ability to trigger a plant's natural defence mechanism (Yedidia *et al.*, 1999). Induced resistance is similar in terms of the gene products involved in systemic acquired resistance (Handelsman and Stabb, 1996).

Antibiosis has been identified as a means of control (Lo, 1998), with the secretion of the following by the antagonist playing an important role: antibiotics, volatile substances and lytic enzymes. Antibiotics such as gliovirin and gliotoxin, produced by *Gliocladium* and *Trichoderma* spp., for example, play an active role in disease suppression (Harman, 2001). More than one antibiotic may be produced by a BCA, lowering pathogen exposure to only one antibiotic. This emphasises the

theory that an interaction between antagonistic microbes may be required to enhance control (Howell, 1987). The potential for pathogen resistance to the antibiotics produced is also reduced (Handelsman and Stabb, 1996). Alkyl pyrones (volatile substances), produced by *T. harzianum*, are also known to suppress disease (Claydon *et al.*, 1987). Disease suppression by *B. subtilis*, could be attributed to the production of antifungal metabolites, which have antibiotic activities which enhance plant health (Leifet *et al.*, 1995; Krebs *et al.*, 1998).

Parasitism or predation of pathogenic fungi is a means of control often employed by mycoparasites (Handelsman and Stabb, 1996). Antagonistic *Trichoderma* and *Gliocladium* spp. are known to grow over the pathogen, coiling around the pathogenic hyphae and thereafter actively attacking them (Benhamou and Chet, 1993; Chambers and Scott, 1995). Parasitism is often associated with other methods of disease suppression, specifically the production of antibiotics (Chambers and Scott, 1995).

1.5.2 PROPOSED MECHANISMS EMPLOYED TO INDUCE GROWTH RESPONSES

The mechanisms employed by plant growth-promoting microorganisms are inconclusive and difficult to determine (Agrios, 1997). Only once these mechanisms are understood, will the application of these microorganisms be more selective.

It is suspected that control mechanisms include:

Tolerance to stress through enhanced root and plant development (Harman, 2000). Enhanced root development improves the area for water and nutrient uptake (Burr and Caesar, 1984; Kapulnik, 1996; Harman, 2000). Increased nutrient uptake is attributed to an increase in root cell permeability (Brown, 1974).

Antagonistic microorganisms **produce antibiotics and other metabolites**, resulting in the control of soil-borne pathogens (Curl and Truelove, 1986).

Niche exclusion and the **control of minor pathogens** are proposed methods of antagonistic behaviour (Windham *et al.,* 1986). This is attributed to out-competing the pathogen for food, minerals and nutrients required for growth, or by means of intercepting signals from the host so that the pathogen is unable to recognise the presence of the root (Fravel, 1992).

The production of growth-regulating factors (Brown, 1974; Lifshifz *et al.*, 1986). Both *Bacillus* and *Trichoderma* spp. produce growth promoting hormones (Burr and Caesar, 1984; Kim-Jeong *et al.*, 1992).

The **mineralisation of bound minerals** for plant use. In clay soils, phosphate (P) is bound and unavailable for plant development (Engelhard, 1989) and microbial activity increases P availability (Anon, 2002).

1.6 ADVANTAGES AND LIMITATIONS ASSOCIATED WITH BIOLOGICAL CONTROL

The advantages associated with biological control have resulted in a more "rational approach" to disease control. Biological control is expected to become an important means of disease control in the future (Agrios, 1997).

1.6.1 ADVANTAGES OF BIOLOGICAL CONTROL

A changing socio-economic climate has increased concerns associated with synthetic chemicals. Chemical residues are commonly detected in food and water sources, affecting the environment and human health (Kerry, 1992; Schwarz, 1992; Hartman, 1996; Lo *et al.*, 1996; Thomas and Willis, 1998).

Growers and manufacturers are more aware of the concerns associated with synthetic chemical usage. The effect of synthetic pesticides in food products for children and infants' food products must now meet a "negligible risk" standard

(Marrone, 1999). Concern about worker safety and inconvenience of interrupted schedules due to re-entry times after chemical applications (Harman, 2000), are considered further advantages for the application of BCAs over chemical use.

The **use of BCAs has proved effective in terms of disease control and stimulated growth responses** (Chang *et al.*, 1986; Cook, 1990; Chet, 1990; Schroth and Becker, 1990). It must be noted that the effect of BCAs is considered not to be specific (Harman, 2000).

BCAs in agricultural systems have the potential to increase nitrogen efficiency, with fertilization being reduced by at least 38% (Harman, 2000).

Organic farming has improved the prospects for BCAs. The demand for a more natural means of agricultural production has resulted in a greater interest and knowledge by farmers, emphasising biological solutions (Harman, 2000).

A problem associated with excessive chemical usage is the **build-up of a pathogen's resistance levels to synthetic chemicals**. The increase in resistance reduces the availability of chemical plant protection agents (Baker, 1983; Kerry, 1992; Hartman, 1996; Lo *et al.*, 1996; Marrone, 1999).

The intensive use of **synthetic chemicals may harm antagonistic microbes** (Mendgen *et al.*, 1992; Lo *et al.*, 1996; Lo *et al.*, 1997), whereas use of antagonists may boost or compliment the natural communities already present.

The **rising costs associated with registration of agrochemicals** is a major incentive (Mendgen *et al.,* 1992). In 1990, the cost of introducing a new BCA over a period of 3 years, cost $5 million in comparison to $80 million associated with chemical registration (exchange rate at present is greater than R7:$1). Although these figures are expressed in dollars, this currency has the largest influence on the world market prices (Woodhead *et al.*,1990).

BCAs can be integrated with fungicides use, as many fungicide-resistant BCA strains exist (Papavizas, 1985; Abd-El-Moity, 1992; Harman, 2001). Chemical and *Trichoderma* seed treatments may also be compatible (Harman, 2000).

Potential compatibility of BCAs will give a wider range of disease control. Small quantities of several BCAs, have been shown to give season-long benefits (Harman, 2000). This is, however, not guaranteed.

Ease of **application of antagonistic microorganisms into the root rhizosphere, as well as the manipulation of the soil environment** has increased interest in BCAs (Nigam and Mukerji, 1988; Rahe, 1988).

A need for a closely **regulated biological control** has been identified (FAO, 1996). This will address public concerns about the use of "living fungi".

Informal education system based on the importance and practical use of biological control, using natural enemies must be addressed (Williamson, 1998). Realisation of the importance of natural enemies in controlling pests will only encourage the use of BCAs.

The **development of computerised models** to determine survival, activity and various levels of interaction of the amended BCAs in the soil and the rhizosphere have also been identified (Papavizas, 1985; Handelsman and Stabb, 1996). This will play a great role in future research for rhizosphere persistent microbes.

1.6.2 LIMITATIONS OF BIOLOGICAL CONTROL

The recognition of potential limitations could only be advantageous in improving future standards of biological control (Thomas and Willis, 1998).

Biological activities are dependent on environmental conditions (Andersch, 1992). The testing of **antagonists are generally limited to controlled environments**. More research is required to determine the effects of biotic and abiotic factors on the activity of BCAs within open environments (Kerry, 1992; Koch, 1999).

The efficiency and **benefits of BCAs are often only realised within an integrated system** (Dent, 1993; Chen *et al.*, 1996). This is especially applicable to high disease pressures (Harman, 2000).

Biological control products are generally associated with a **lack of economic advantages** (Klob, 1992). The product mark-up of organic food is often higher than that of chemically treated produce. Many consumers are not willing to pay the extra mark-up, and a producers' economic returns are therefore lower. Biofungicides also have a higher market price than the alternative chemical product (Orr and Baker, 1997a; Marrone, 1999; Harman, 2000).

Biological control products must satisfy the same parameters as chemical products, providing cost effective, blemish-free produce (Klob, 1992; Schwarz, 1992). Many companies see no reason to commercialize BCAs when a highly effective synthetic chemical product exists (Harman, 2000).

Assessing the impact on natural enemies and their implementation in production systems has been poorly supported. Unrestrained use of chemicals has led to pathogen resistance, as well as non-target effects on natural enemies. Careful planning and knowledge of the biology and the mechanism of action of the BCA and pesticide are essential (Orr and Baker, 1997a).

The application of antagonists is **dependent on the accurate and timely detection of symptoms** (Hartman, 1996). BCAs are considered to be preventative and are less effective against systemic diseases (Orr and Baker, 1997a; Harman, 2000).

Little conclusive evidence is known about the specific requirements and capabilities of biological products. This results in unreliable and poor performance associated with BCAs (Kapulnik, 1996; Koch, 1999). A better understanding of biological control will widen its potential for effective use (Handelsman and Stabb, 1996). Inconsistent results are not only unique to BCAs, but are common to agrochemicals too (Orr and Baker, 1997b).

It is accepted that BCA use is **less dramatic or quick-acting than agrochemicals** (Orr and Baker, 1997a). The benefits of BCAs are only realised in the long-term or under stress (Harman, 2000).

Potential use of BCAs has to not only **compete with synthetic chemicals, but also with other disease control alternatives** (Wilson and Wisniewski, 1992).

A **narrow biological spectrum of beneficial organisms** exists. Presently research is limited to cultured BCAs (Handelsman and Stabb, 1996).

There is concern about the **public's reaction to food treated with living fungicides**. These are, however, based on potential risks, while chemical control risks are based on existing knowledge (Curl and Truelove, 1986; Lynch, 1992).

There is **public concern about genetically modified microorganisms** (Klob, 1992). Genetic manipulation of microorganisms, although it provides greater future potential, poses a problem in terms of registration (Dent, 1993).

Little consideration is given to the **potential risks associated with "exotic" BCAs on non-target hosts and the conservation and preservation of biodiversities** (Thomas and Willis, 1998). Extensive testing is done to determine the risks of BCAs on target and non-target hosts.

Some **beneficial microorganisms produce antibiotics**, these have the potential to be allergenic (Schroth, 1992). Once the potential problems and risks have been determined, there is little to be concerned about in terms of the release of BCAs. It must be stressed that the production of antibiotics is not the only means of activity expressed by BCAs (Harman, 2000)

More research is needed into the mechanism by which beneficial (antagonistic) microorganisms can be delivered into the soil at an optimum inoculation level that will ensure survival (Kerry, 1992; Dandurand and Knudsen, 1993; Kapulnik, 1996; Harman, 2000). Survival and colonization of the rhizosphere will ensure disease control and plant growth stimulation.

Successful inoculation is **dependent on soil characteristics and existing cultivation measures** (Mendgen *et al,* 1992). It has been proposed that disease suppression may not be due to rhizosphere activity, but due to the microbial community change that occurs through inoculation (Handelsman and Stabb, 1996).

Fungal and bacterial antagonists must be rhizosphere competent (Cook, 1993). Effective control is limited by a competitive soil environment (Harman, 2000).

The **registration of potential BCAs** is inexpensive and simple in comparison to chemical registration (Harman, 2000). However, it requires extensive screening and testing which is time-consuming.

1.7 FUTURE OF BIOLOGICAL CONTROL

Early pesticides were highly toxic with broad spectrum and persistent toxic derivatives. The introduction of *B. thuriengensis* (Bt)-based pesticides provided a non-toxic, host specific biological alternative with low persistence in agro-ecosystems (Dent, 1993). Wilson and Wisniewski (1992) predicted that the agricultural chemical industry as we know it today will begin to disappear and biological products will become cost-competitive. If this prediction holds true, there is a wide and promising future for biological control. Since 1960, there has also been an increase in public awareness of the potential risks of synthetic chemicals to the environment. Numerous government agencies control the use of pesticides, suggesting that cultural practices be implemented instead (Baker, 1992). Countries such as S.A., U.S.A., Australia, Canada and New Zealand (N.Z.) are slowly realising that biological control could provide a safe and environmentally sustainable alternative to synthetic chemical use (Cruttwell-McFayden, 1998; Harman, 2000).

The future of biological disease control and plant growth stimulation is based on a greater understanding of rhizosphere and phylloplane activities. Research findings into the development of commercial biological products has grown and numerous biological formulations are now available (Jutsum, 1988; Schroth and Becker, 1990). Recent developments in biological control have emphasised the potential advantages associated with the compatibility of several BCAs (Dandurand and Knudsen, 1993). The future, however, is in the development of widey adapted BCAs which demonstrate a wide range of activity (Harman, 2000).

Biological control measures may not replace chemicals from their dominant role in disease control, especially as chemical research is adopting the production of safer synthetic chemicals (Cremlyn, 1991). Organic farming will emphasise the implementation of microorganisms for disease control. Many researchers have also predicted that the future of biological control lies in an integrated approach to disease control.

Weeds, insects, plant diseases and other plant constraints are on the increase due to the intensification of production systems (Thomas and Waage, 1996). With the increase in cropping or production areas, pests are "forced" to find alternate hosts posing a threat to crops. The demand for better varieties has also resulted in an increase in the movement of plant material and exotic pests and diseases into production areas. Alternative methods of suppression must be explored for the future.

1.8 CONCLUSIONS

Synthetic chemicals will always play a pivotal role in disease control, however, the implementation of alternatives such as an integrated or biological control system must be considered (Woodhead *et al.*, 1990). A suppressive soil supports potential BCAs. Careful selection of agents, a thorough understanding of the ecology of the agent, the target and the best means of efficient implementation require future research. This must be based on collaboration between researchers, producers and industry (Kerry, 1992). Various field trials have already proved that biological control offers a window of opportunity as an alternative to synthetic products (Dent, 1993). Biological control itself may not develop to a standard of control achieved with synthetic chemicals. A need for an alternative to disease control has been identified repeatedly by agriculturalists and horticulturalists. Biological disease control is, therefore, a promising strategy for the management of soil-borne and foliar pathogens, as well as enhancing overall plant performance in a wide range of crops (Lo *et al.*, 1997).

1.9 REFERENCES

Abd-El-Moity, T. 1992. The use of *Trichoderma* spp. to control soilborne plant pathogens in Egypt. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 255-258.

Ahmad, I., J. Bissett and D. Malloch. 1995. Effect of phosphinothricin on nitrogen metabolism of *Trichoderma* species and its implications for their control of phytopathogenic fungi. Pesticide Biochemistry and Physiology **53:** 49-59.

Agrios, G. 1997. Plant Pathology, 4th edition. Academic Press, California: United States of America.

Andersch, W. 1992. Production of fungi as crop protection agents. In: Pflanzenschutz-Nachrichten Bayer 45/1992 (63). Bayer AG, Geschaftsbereich Pflanzenshutz, Leverkusen: Germany. p. 129-142.

Anon, 2002. Microbial Solutions: product range. http://www.microbial.co.za (accessed 24 July 2003).

Baker, K.F. 1983. The future of biological and cultural control of plant diseases. In: T. Kommendahl and P.H. Williams (eds). Challenging problems in plant health. The American Phytopathology Society, Minnesota: United States of America. p. 422-430.

Baker, C.J., J.R. Stavely and N. Mock. 1985. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. Plant Disease **69:** 770-772.

Baker, R. 1992. Biological control of diseases of crops grown in covered and environmentally controlled structures. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 231-241.

Bazin, M.J., P. Markham, and E.M. Scott. 1990. Population dynamics and rhizosphere interactions. In: J.M Lynch (ed). The rhizosphere. John Wiley & Son, Chichester: United Kingdom. p. 99-128.

Benhamou, N. and I. Chet. 1993. Hyphal interactions between *Trichoderma harzianum* and *Rhizoctonia solani*: ultrastructure and gold cytochemistry of the mycoparasitic process. Phytopathology **83:** 1062-1071.

Berger, F.D., H. Li, D. White, R. Frazer and C. Leifert. 1996. Effect of pathogen inoculum, antagonist density and plant species on biological control of *Phytophthora* and *Pythium* damping-off by *Bacillus subtilis* Cot 1 in high-humidity fogging glasshouses. Phytopathology **86:** 428-433.

Brown, M.E. 1974. Seed and root bacterization. Annual Review of Phytopathology **12:** 181-197.

Burr, T.J. and A. Ceaser. 1984. Beneficial plant bacteria. In: B.V. Conger (ed). Critical reviews in plant science, Volume 2:1. CRC Press, Florida: United States of America. p. 1-20.

Callan, N.W., D.E. Mathre and J.B. Miller. 1990. Bio-priming seed treatment by biological control of *Pythium ultimum* pre-emergence damping-off in *sh*2 sweet corn. Plant Disease **74:** 368.

Chambers, S.M. and E.S. Scott. 1995. *In vitro* antagonism of *Phytophthora cinnamomi* and *P. citricola* by isolates of *Trichoderma* spp. and *Gliocladium virens.* Journal of Phytopathology **143:** 471-477.

Chang, Y.C., Y.C. Chang, R. Baker, O. Kliefeld, and I. Chet. 1986. Increased growth of plants in the presence of the biological control agent, *Trichoderma harzianum.* Plant Disease **70:** 145-148.

Chen, J., L.M. Jacobson, J. Handelsman and R.M. Goodman. 1996. Compatibiliy of systemic acquired resistance and microbial biocontrol for suppression of plant disease in laboratory assays. Molecular Ecology **5:** 73-80.

Chet, I. 1990. Biological control with fungal antagonists and soil treatments. In: D. Hornby (ed). Biological control of soil-borne plant pathogens. C.A.B. International, Wallingford: United Kingdom. p.15-26.

Chet, I. and R. Baker. 1980. Induction of suppressiveness to *Rhizoctonia solani* in soil. Phytopathology **70:** 994-998.

Claydon, N., M. Allan, J.R. Hanson and A.G. Avent. 1987. Antifungal alkyl pyrones of *Trichoderma harzianum.* Transactions of the British Mycological Society **88:** 503-513.

Cliquet, S. and R.J. Scheffer. 1997. Influence of culture conditions on growth and survival of conidia of *Trichoderma* spp. coated on seeds. Biocontrol Science and Technology **7:** 171-181.

Conway, K.E. 1986. Use of fluid-drilling gels to deliver biological control agents to soil. Plant Disease **70:** 835.

Cook, R.J. 1988. Management of the environment for the control of pathogens. In: R.K.S.Wood, F.R.S. Way and M.J. Way (eds). Biological control of pests, pathogens and weeds: developments and prospects. Philosophical Transactions of the Royal Society of London, Volume 318:1189. Royal Society, London: United Kingdom. p. 173.

Cook, R.J. 1990. Twenty-five years of progress towards biological control. In: D. Hornby (ed). Biological control of soil-borne plant pathogens. C.A.B International, Wallingford: United Kingdom. p. 1-14.

Cook, R.J. 1993. Making greater use of introduced microorganisms for biological control of plant pathogens. Annual Review of Phytopathology **31:** 53-80.

Cremlyn, R.J. 1991. Agrochemicals: preparation and mode of action. John Wiley & Sons, New York: United States of America. p. 361-372.

Cruttwell-McFayden, R.E. 1998. Biological control of weeds. Annual Review of Entomology **43:** 369-393.

Curl, E.A. and B. Truelove. 1986. The rhizoshere. Springer-Verlag, Berlin: Germany.

Dandurand, L.M. and G.R. Knudsen. 1993. Influence of *Pseudomnas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma harzianum* in the spermosphere and rhizosphere of pea. Phytopathology **83:** 265-270.

Deacon, J.W. and L.A. Berry. 1992. Modes of action of mycoparasites in relation to biocontrol of soilborne plant pathogens. In: E.C. Tjomas, G.C. Papavizas and R.J.Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 157-164.

De Ceuster, T.J.J. and H.A.J. Hoitink. 1999. Using compost to control plant diseases. BioCycle, June 1999. p. 61-64.

Dent, D.R. 1993. The use of *Bacillus thuringiensis* as an insecticide. In: D.G. Jones (ed.) Exploitation of micro-organisms. Chapman & Hall, London: United Kingdom. p. 19-32.

Engelhard, A.W. 1989. Soilborne plant pathogens: management of diseases with macro- and micro-nutrients. American Phytopathology Society, Minnesota: United States of America.

Fiddaman, P.J. and S. Rossall. 1995. Selection of bacterial antagonists for the biological control of *Rhizoctonia solani* in oilseed rape (*Brassica napus*). Plant Pathology **44:** 695-703.

Food and Agriculture Organization of the United Nations, 1997. Code of conduct for the import and release of exotic biological control agents, November 1996. International Standards for Phytosanitation Measures, Publication no. 3, FAO, Rome: Italy.

Fravel, D.R. 1992. Systems for efficient delivery of microbial biocontrol agents to soil. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 399-413.

Grant, Z. 1993. Integrated pest management in the golf course industry: a case study and some general considerations. Golf Course Superintendents Association of America, Lawrence: United States of America.

Guetsky, R., D. Shteinberg, Y. Elad and A. Dinoor. 2001. Combining biocontrol agents to reduce the variability of biological control. Phytopathology **91:** 621-627.

Handelsman, J. and E.V. Stabb. 1996. Biocontrol of soilborne plant pathogens. The Plant Cell **8:** 1855-1869.

Harman, G.E. and A.G. Taylor. 1988. Improved seedling performance by integration of biological control agents at favourable pH levels with solid matrix priming. Phytopathology **78:** 520.

Harman, G.E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease **84:** 377-393.

Harman. G.E. 2001. *Trichoderma* spp., including *T. harzianum, T. viride, T. koningii, T. hamatum* and other spp. Cornell University: Geneva: United States of America.
www.nysaes.cornell.edu/ent/biocontrol/pathogens/trichoderma.

Hartman, J.P. 1996. Integrated production: a new approach to environmental and consumer needs. In: Integrated pest management and soilborne plant diseases. Interdisciplinary meeting/seminar by the Plant Protection Research Institute, Vredenberg Research Centre, Stellenbosch: South Africa. p. 4-13.

Hazen, B.E. 1989. Biological control of brown rot of postharvest peaches with *Bacillus subtilis.* Journal of Agricultural Food Chemistry **36:** 366.

Howell, C.R. 1987. Relevance of mycoparasitism in the biological control of *Rhizoctonia solani* by *Gliocladium virens*. Phytopathology **77:** 992-994.

Jutsum, A.R. 1988. Commercial application of biological control. In: R.K.S. Wood, F.R.S. Way and M.J. Way (eds). Biological control of pests, pathogens and weeds: developments and prospects. Philosophical Transactions of the Royal Society of London, Volume 318:1189. Royal Society, London: United Kingdom. p. 252.

Kapulnik, Y. 1996. Plant growth promotion by rhizosphere bacteria. In: Y. Wisel, A. Eshel and U. Kafkafi (eds). Plant roots: the hidden half, 2nd edition. Marcel Dekker, New York: United States of America. p. 769-781.

Kerr, A. 1980. Biological control of crown gall through production of grocin. Plant Disease **64:** 25.

Kerry, B.R. 1992. Biological control of soil-borne pests and diseases. In: Biological control and integrated crop protection: towards environmentally safe agriculture. Pudoc Scientific Publishers, Wageningen: Holland. p.117-123.

Kim, D., R.J. Cook and D.M. Weller. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology **87:** 551-558.

Kim-Jeong, G., B.R. Jeong and J.M. Brown. 1992. Population transfer along transplanted seedlings and lack of plant growth promotion by three *Trichoderma harzianum* strains. Acta Horticulturae **319:** 419-424.

Kleifeld, O. and I. Chet. 1992. *Trichoderma harzianum*: interaction with plants and effect on growth response. Plant and Soil **144:** 267-272.

Klob, F. 1992. Status of biocontrols in integrated crop protection in Europe. In: Pflanzenschutz-Nachrichten Bayer 45/1992 (63). Bayer AG, Geschaftsbereich Pflanzenshutz, Leverkusen: Germany. p. 99-112.

Knudsen, G.R., D.J. Eschen, L.M. Dandurand and L. Bin. 1991. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum*. Plant Disease **75:** 466-470.

Koch, E. 1999. Evaluation of commercial products for microbial control of soil-borne plant diseases. Crop Protection **18:** 119-125.

Krebs, B., B. Höding, S. Kübart, M. Alemayehu Workie, H. Junge, G. Schmiedeknecht, R. Grosch, H. Boschow and M. Hevesi. 1998. Use of *Bacillus subtilis* as a biocontrol agent.I. Activities and characterization of *Bacillus subtilis* strains. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz **105:** 181-197.

Leifet, C., H. Li, S. Chidburee, S. Hampson, S .Workmann, D. Sigree, H.A.S. Epton and A. Harbour. 1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. Journal of Applied Bacteriology **78:** 97-108.

Lewis, J.A., R.P. Larkin and D.L. Rogers. 1998. A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in a soilless mix. Plant Disease **82:** 501-506.

Lo, C.T., E.B. Nelson and G.E. Harman. 1996. Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum.* Plant Disease **80:** 736-741.

Lo, C.T., E.B. Nelson and G.E. Harman. 1997. Improved biocontrol efficacy of *Trichoderma harzianum* 1295-22 for foliar phases of turf diseases by use of spray applications. Plant Disease **81:** 1132-1138.

Lo, C.T. 1998. General mechanisms of action of microbial biocontrol agents. Plant Pathology Bulletin **7:** 155-166.

Lutchmeah, R.S. and R.C. Cooke. 1985. Pelleting of seed with the antagonist *Pythium oligandrum* for biological control of damping off. Plant Pathology **34:** 528-531.

Lynch, J.M. 1992. Environmental implications of the release of biocontrol agents. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 389.

Mandeel, Q. and R. Baker. 1991. Mechanisms involved in biological control of *Fusarium* wilt of cucumber with strains of nonpathogenic *Fusarium oxysporum.* Phytopathology **81:** 462-469.

Marrone, P.G. 1999. Microbial pesticides and natural products as alternatives. Outlook on Agriculture **28:**149-154.

Mendgen, K., A. Schiewe, and C. Falconi. 1992. Biological control of plant diseases. In: *Pflanzenschutz-Nachrichten* Bayer 45/1992 (63). Bayer AG, Geschaftsbereich Pflanzenshutz, Leverkusen: Germany. p. 5-14.

Nebec, S. 1997. Longevity of microbial biocontrol agents in a planting mix amended with *Glomus intraradices.* Biocontrol Science and Technology **7:** 183-192.

Nigam, N. and K.G. Mukerji. 1988. Biological control - concepts and practices. In: K.G. Mukerji and K.L. Garg (eds). Biocontrol of plant diseases, volume 1. CRC Press, Florida: United States of America. p. 1-14.

Orr, D. and J. Baker. 1997a. Biological control: purchasing natural enemies. Department of Entomology: North Carolina State University. ipmwww.ncsu.edu/biocontrol/3a.htm

Orr, D. and J. Baker. 1997b. Biological control: application of natural enemies. Department of Entomology: North Carolina State University. ipmwww.ncsu.edu/biocontrol/3b.htm

Ousley, M.A., J.M. Lynch and J.M. Whipps. 1993. Effect of *Trichoderma* on plant growth: a balance between inhibition and growth promotion. Microbial Ecology **26:** 277-285.

Ousley, M.A., J.M. Lynch and J.M. Whipps. 1994. The effects of addition of *Trichoderma* inocula on flowering and shoot growth of bedding plants. Scientia Horticulturae **59:** 147-155.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Annual Review of Phytopathology **23:** 23-54.

Peng, G., J.C. Sutton and P.G. Kevan. 1992. Effectiveness of honey bees for applying the biocontrol agent *Gliocladium roseum* to strawberry flowers to suppress *Botrytis cinerea.* Canadian Journal of Plant Pathology **14:** 117-129.

Prakash, M.G., K.V. Gopal, M. Anandaraj and Y.R. Sarma. 1999. Evaluation of four substrates for mass multiplication of fungal biocontrol agents *Trichoderma harzianum* and *T. virens*. Journal of Spices and Aromatic Crops **8:** 207-210.

Rahe, J.E. 1988. Biological control, genetic engineering, and crop disease management. In: K.G. Mukerji and K.L. Garg (eds). Biocontrol of plant diseases, volume II. CRC Press, Florida: United States of America. p. 26.

Raviv, M., B.Z. Zaidman and Y. Kapulnik. 1998. The use of compost as a peat substitute for organic vegetable transplant production. Compost Science and Utilization **6:** 46-52.

Rodham, D.K., Y. Wang, J.B. Cantwell, P.D. Winn and J. Foundling. 1999. Formulating microbial biocontrol agents. Pesticide Science **55:** 340-342.

Samuels, G.J. 1996. *Trichoderma*: a review of biology and systematics of the genus. Mycological Research **100:** 923-935.

Schippers, B. 1992. Prospects for management of natural suppressiveness to control soilborne pathogens. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 21.

Schroth, M.N. and J.O. Becker. 1990. Concepts of ecological and physiological activities of rhizobacteria related to biological control and plant growth promotion. In: D. Hornby (ed). Biological control of soil-borne plant pathogens. CAB International, Wallingford: United Kingdom. p. 389-414.

Schroth, M.N. 1992. Risks of releasing wild-type and genetically engineered biocontrol organisms into the ecosystem. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 378-379.

Schwarz, M.R. 1992. Biological and integrated pest and disease management in the United States of America. In: Pflanzenschutz-Nachrichten Bayer 45/1992 (63). Bayer AG, Geschaftsbereich Pflanzenshutz, Leverkusen: Germany. p. 73-82.

Singh, J. and J.L. Faull. 1988. Anatagonism and biological control. In: K.G. Mukerji and K.L. Garg (eds). Biocontrol of plant diseases, volume II. CRC Press, Florida: United States of America. p. 167-175.

Thomas, M. and J. Waage. 1996. Integration of biological control and host plant resistance breeding: a scientific and literature review. Technical Centre for Agriculture and Rural Cooperation (ACP-EU), Wageningen: Netherlands. p.1-8.

Thomas, M.B. and A.J. Willis. 1998. Biocontrol-risky but necessary? TREE **13:** 325-329.

Tjomas, E.C. 1992. Selective elimination of soilborne plant pathogens and enhancement of antagonists by steaming sublethal fumigation and soil solarization. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 9.

Tronsmo, A. 1992. Leaf and blossom epiphytes and endophytes as biological control agents. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 48.

Tu, J.C. 1992. Biological control of root rot diseases in peas. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 318.

Waage, J.K. and D.J. Greathead. 1988. Biological control: challenges and opportunities. In: R.K.S. Wood, F.R.S. Way and M.J. Way. Biological control of pests, pathogens and weeds: developments and prospects. Philosophical Transactions of the Royal Society of London, 318: 1189. Royal Society, London: United Kingdom. p. 111-128.

Williamson, S. 1998. Understanding natural enemies; a review of training and information in the practical use of biological control. Biocontrol News and Information **19:** 117-125.

Wilson, C.L. and M.E. Wisniewski. 1992. Future alternatives to synthetic fungicides for the control of postharvest diseases. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America.

p.133-137.

Windham, M.T., Y. Elad and R. Baker. 1986. A mechanism of increased growth induced by *Trichoderma* spp. Phytopathology **76:** 518-521.

Woodhead, S.H., A.L. O'Leary, D.J. O'Leary, and S.C. Rabatin. 1990. Discovery, development and registration of a biocontrol agent from an industrial perspective. Canadian Journal of Plant Pathology **12:** 328-331.

York, K. 1996. Take-all patch in cereals and amenity turf. International Turfgrass Bulletin **193:** 29-30.

Yuen, G.Y., M.L. Craig and L.J. Giesler. 1994. Biological control of *Rhizoctonia solani* on tall fescue using fungal antagonists. Plant Disease **78:** 118-123.

CHAPTER 2

COMMON DISEASES ASSOCIATED WITH TURF AND PASTURE GRASSES

2.1 INTRODUCTION

Disease is defined as an abnormal condition of plants, resulting from alterations in physiological processes and morphological development caused by adverse factors (Agrios, 1997). Various abiotic and biotic factors therefore contribute to disease development. In terms of biotic diseases, the disease-inciting microorganisms at a specific site are usually determined by the host species present. In terms of pasture and turf diseases in South Africa (S.A.), there is limited information at present. Most greenkeepers and farmers have established their knowledge from trial and error. The information sources that contribute to this chapter are therefore mostly from New Zealand (N.Z.), Australia and the United States (U.S.).

Emphasis has been placed, of late, on the selection of new and improved turfgrass species. These must not only be drought and shade tolerant but also tolerant to heavy traffic. Most importantly they must be disease tolerant, as disease detracts from the aesthetic value and often limits the use or playing quality of the stand (Baldwin, 1990). Disease tolerance of pasture grasses is also important, as severe infections limit yields, reduce animal performance and may even result in poisoning of grazing livestock (Hall, 1992). Although the importance of diseases on pastures is not realized at present, the increased use of pastures and the use of improved varieties and mixes will place greater emphasis on the impact of disease on pasture productivity and persistence. The prevention of diseases on turf and pasture grasses has been a long-standing issue that has not yet been successfully resolved.

The most common diseases of grasslands are usually fungal related (Couch, 1995; Peters and Shaw, 1996). Theoretically, the best means of controlling any pathogen, lies in the prevention of conditions that are most conducive to disease development. At present the predominant means of controlling plant diseases is with the use of agrochemicals. Agrochemicals have, however, sustained much criticism and many diseases have developed fungicide resistant strains. Emphasis has therefore, been placed on the use of antagonistic microorganisms as biocontrol agents (Chapter 1). This concept is largely unexplored for pathogens of pasture and turfgrasses. Cultural practices in disease management are also important, simple and cost effective to implement. An integrated approach to disease control, using agrochemicals, cultural practices and biocontrol agents, appears to be the trend in many parts of the world.

This chapter serves as an overview of fungal diseases encountered by pasture farmers and greenkeepers within the areas surrounding Pietermaritzburg, KwaZulu Natal. Only those diseases identified in a survey, conducted in the area (see Chapter 3), as severe or occasionally encountered, are mentioned. The diseases presented here have been summarised into a table defining the causal agent(s), common disease symptoms observed, disease cycle, common pasture and turfgrass hosts, control measures and relative importance of the disease. The relevant references have been included in a column as further reference material. A table summarising plant parasitic nematodes has also been included, as the importance of these disease-inciting organisms is not realized to its full potential.

2.2 FUNGAL DISEASES OF TURF AND PASTURE GRASSES

Table 2.1 Summary table of *Colletotrichum graminicola* (Ces.) Wils., commonly referred to as ANTHRACNOSE

Symptoms[a]	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• patches of light greenish/yellow grass, 1-15cm in diameter • entire patches become yellow (brick red under cool conditions) then finally tan-brown • individual leaf symptoms appear as yellow tips of older leaves, turning tan-brown and progressing to the leaf sheath • close examination of diseased leaves reveals acervuli • infected plant stems can be pulled from the crowns • in spring/early summer a basal rot appears as a dark-brown/black discolouration at the base the leaf sheath and stems, and in severe cases on the roots as well	• basal rot is associated with cool (15-24°C), wet conditions • leaf blight is associated with warm temperatures (29-35°C), extended leaf wetness and high humidity • a high population of root, crown and leaf feeding insects; nematodes; parasitism by pathogenic fungi, unsuitable soil fertility; high temperatures (30-35°C); cold injury; poor oxygen levels; moisture stress and soil compaction predisposes plants to infection.	• pathogen overwinters in living plant tissues, leaf litter and/or thatch as hyphae and conidia • infection and colonization is associated with biotic and abiotic stresses	• cool- and warm-season grasses. • severe cases limited to: *Poa annua* L.; *Agrostis* spp., *Lolium perenne* L. and *Festuca rubra* L. • other hosts include: *Agrostis canina* L.; *Agrostis palustris* Huds.; *Agrostis tenuis* Sibth *A. canina* L.; *Cynodon dactylon* L; *Echinichloa crusgalli* (L.) Beauv.; *Festuca ovina* L.; *F. arundinacea* Shreb.; *Lolium multiflorum* Lam.; *Poa pratensis* L. and *Zoysia japonica* Steud.	***Resistance***: susceptible grasses are *P. annua; Agrostis* spp.; *L. perenne* and *F. rubra*. Avoid planting these grasses in areas with prominent outbreaks. Where possible, implement mixed cultivar planting to increase the genetic diversity of the stand. Resistance breeding programmes are ongoing. ***Cultural control***: the aim is to reduce inoculum and unnecessary plant stress • avoid thatch accumulation greater that 1.3-2cm thick; • maintain field capacity (irrigate to 250-300mm) through light irrigation during the hottest day periods; • core to increase water infiltration and combat soil compaction; • maintain soil nutrient status, especially phosphorus (P) and potassium (K); • remove infected plant material, through burning; • optimise grazing management, in terms of timing and intensity and the management of waterlogged soils and • quarantine infected areas if feasible and control plant movement between countries and geographical areas. ***Biological control***: naturally occurring antagonistic bacteria have been isolated from tropical pastures ***Chemical control***[b]: infection is limited to older leaves - fungicide applications are therefore not warranted. • the following active ingredients are effective: folpet/sulphur; mancozeb and zineb, and benomyl as a seed treatment; • nematicide and pesticide applications will control disease entry points	• widespread on cool- and warm-season grasses • disease detracts from the aesthetic value and potential use of the area • secondary disease with *Exserohilum*-incited diseases	Davis and Grof, 1994; Davis and Irwin, 1994; Trutmann, 1994 a & b; Couch, 1995 Krause *et al.*, 1996; Nel *et al.*, 1999

a Symptoms vary with the grass species infected and prevailing weather conditions

b A table of fungicides : common names and active ingredients and the diseases that they control is shown in Appendix 1

Table 2.2 Summary table of *Magnaporthe grisea* (Herbert) Barr. (*Pyricularia grisea* (Cooke) Sacc.), commonly referred to as BLAST[c]

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• disease affects the leaves and stems • initial symptoms appear as tiny oval, brown or grey/green, watersoaked spots • as conditions become more favourable the spots enlarge into greyish/brown, round/ oblong lesions (1.5mm x 1.8mm), with dark-brown to brownish-purple margins and chlorotic halos. • infected seedlings are flaccid and water-soaked, with a blue-grey appearance • on mature tillering grass, chlorotic halos coalesce, killing the infected leaf or causing breakages at the infected nodes • warm, humid conditions result in sporulation, forming a grey mould over the lesions • in severe outbreaks a "scorched" or "blighted-twisted" stand appearance is common • within one week from the initial symptoms, thick mats of dead, straw/tan coloured patches appear • small grains inter-planted with pastures are also susceptible; symptoms differ but include severe water-soaking and large areas of necrotic tissues; individual lesions are difficult to discern.	• disease is prevalent during moderate to warm temperature (25-30°C), 90% humidity and extended periods of leaf wetness • high nitrogen fertilization and exposure to stress factors, such as the incorrect use of herbicide sprays, soil compaction and water stress predisposes plants to disease	• pathogen overwinters as dormant mycelia and free conidia on alternate hosts and plant residues • under suitable climatic conditions lesions produce conidiophores bare conidia, which are disseminated by wind, water and traffic; infected seeds also spread disease • the conidia adhere to the suitable host surface with a sticky mucilage, and after several days of moist conditions germinate and penetrate the host directly by appressoria or the natural plant openings • within the tissues, infection is both inter- and intra-cellularly, but vascular tissues are rarely invaded • new water-soaked lesions appear within 3 days after inoculation, under optimal conditions • primary infection of leaves occurs with the advent spring rains; with disease incidences increasing throughout the season	• wide spread on cool - and warm - season turf and pasture grasses • severe cases occur on *Stenotaphrum secundatum* (Walt.) Kuntze and *Lolium* spp. • other grass hosts include: *Cynodon* spp., *Eremochloa* spp., *Paspalum* spp. and *Digitaria sanguinalis* (L.) Scopoli and the cool-season grasses: *Agrostis* spp., *and Festuca* spp. • Gramineous hosts include: *Zea mays* L., *Avena sativa* L., *Triticum aestivum* L. and *Oryza sativa* L.	***Resistance***: turf and pasture cultivars vary in their levels of resistance • when disease incidences are low, plant resistant or moderately resistant cultivars ***Cultural practices***: best control achieved when accompied by resistant cultivars • implement strict management, avoiding drought-like conditions, excessive leaf wetness, soil compaction and stress induced by herbicide applications • irrigate between sunset and sunrise to reduce the leaf wetness period • avoid excessive nitrogen (N) applications during wet months; applications should be split to support an adequate rate of growth ***Chemical control***: varying levels of effectiveness are encountered • systemic fungicides limit host penetration • fungicides with the following active ingredients prove effective against leaf diseases: chlorothaloni, copper ammonium carbonate, copper hydroxide, copper oxychloride, copper oxychloride/sulphur, folpet/sulphur, mancozeb and zineb	• disease spread is rapid, lesions appear 4-5 days after infection; physiological races of the pathogen limit spread • in the U. S. turfgrass losses of 90% or more have been reported, and blast poses a serious threat to the 8 million hectares of annual pastures • outbreaks have occurred in Africa, Asia, Australia, Caribbean, Europe and South America • in S.A., losses to winter ryegrass pastures have occurred	Ranganathaiah and Mathur, 1978; Yaegashi and Udagawa, 1978; Trevathan, 1982; Atilano, 1983; Hall, 1992; Kurschner *et al.*, 1992; Landschoot and Hoyland, 1992; Smiley *et al.*, 1992; Trevathan *et al.*, 1994; Couch, 1995; Krause *et al.*, 1996; Agios, 1997; Fraser *et al.*, 1999a; Nel *et al.*, 1999; Uddin *et al.*, 1999; Long *et al.*, 2000; Uddin *et al.*, 2003

[c] Blast is also referred to as Grey leaf spot (GLS), a disease destructive on both turf and pasture grasses

Table 2.3 Summary table of *Sclerotinia homeocarpa*[d] F.T. Bennett, commonly referred to as DOLLAR SPOT[e]

Symptoms[f]	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• mostly affects the foliage of turfgrasses • on a short turf, symptoms appear as small, circular, sunken, bleached patches (2.5cm in diameter) • on a longer turf/coarser textured turfgrass, the infested area is blighted and straw-coloured, with large and irregular shaped patches (15cm-3.5m in diameter) • if blighted areas go untreated they will coalesce to form large bleached patches • morning dew and high humidity results in a cobweb-like white mycelial growth over the infected area; disappearing as humidity drops and grass dries • individual leaf blades have small chlorotic lesions, which become water-soaked and straw-coloured with reddish-brown margins; lesions enlarge to extend across the diameter of the leaf • pathogen may produce a toxic metabolite to grass roots; roots appear short and thick with little/no root hair formation; eventually becoming brown and withered. Adventitious root development is encouraged	• disease is prevalent under cool (16-18°C), damp/wet conditions (spring or autumn) • optimal conditions for fungal growth are 21-27°C and 85% humidity • outbreaks are associated with: heavy thatch (>1.3cm); low moisture conditions (75% field capacity) and low-fertility soils (ambiguity exists with disease occurrence in association with soil N fertility)	• pathogen overwinters as sclerotia or dormant mycelia in the crowns and roots of living plant material • under suitable conditions these structures germinate, resuming growth giving rise to conidia and ascospores • infection of plant material occurs when mycelia grow through wounded plant material, via the stomata or via direct penetration of the intact plant tissues • primarily disease spread is by mycelial colonization of healthy tissues • long distance dispersal is by relocating diseased plant material and heavy traffic	• disease is common to all turfgrass species • susceptible species include *Agrostis* spp.; *C. dactylon*; *Eremochloa ophiuroides* (Munro) Hack.; *Festuca ovina* L.; *F. rubra*; *F. arundinacea*; *Lolium perennne*; *Paspalum notatum* Flugge; *Poa* spp., *Zoysia japonica* and *Z. tenuifolia* Willd. • Gramineous hosts also exist	***Resistance***: resistant grass cultivars available • cultivars showing higher shoot density are more prone to outbreaks ***Cultural control***: best results obtained together with a suitable fungicide programme • disperse dew or guttation water in the early mornings by raking • remove thatch • promote removal of the leaf tips; in turfgrasses maintain the area at a low mowing height and in pastures graze to remove infected leaf tips • implement strict management, ensuring the correct nutrient status of the stand, especially N and K; avoid moisture-stress by maintaining 75% field capacity at all times; correct or minimise soil compaction through coring; ensure the correct seeding rates and final plant densities ***Biological control:***: recorded with applications of *Trichoderma harzianum* strain 1295-22, as either a spray or broadcast granule application ***Chemical control:*** fungicide applications in the early stages of development will delay or prevent further invasions • dicarboximide fungicides and those with the following active ingredients give satisfactory control: anilazine, chlorothalonil, iprodione, propiconazole, thiabenazole and triadimefon; good or optimal control is achieved with cadmium-containing fungicides (banned in S.A.) and fungicides with the active ingredients thiram and daconil • benlate is used on turf as a soil drench and applications should be preceded by coring; biotypes of the fungus may show resistance; if present, use cultural control methods or change to another registered fungicide	• widespread and destructive on turfgrasses • outbreaks have been reported in Australia, British Isles, Europe, Japan, N.Z. and Northern America • in S.A. the disease is encountered on golf greens, As a disease on pasture grasses little has been recorded	Bloom and Couch, 1960; Vengris and Torello, 1982; Burpee and Goulty, 1986; Brede, 1991; Turgeon, 1991; Smiley *et al.*, 1992; Stahnke, 1993; Couch, 1995; Emmons 1995; Krause *et al.*, 1996; Schroeder and Sprague, 1996; Laing, 1997; Lo *et al.*, 1997; Nel *et al.*,1999

d The pathogen may belong in the genus *Lanzia* or *Moellerodiscus* (Turgeon, 1991)
e Dollar spot is also referred to as *Sclerotinia* dollar spot
f Symptoms vary with management practices implemented and the host plant infected

Table 2.4 Summary table of *Sclerophthora macrospora* (Sacc.) Thirum, commonly referred to as DOWNY MILDEW[g]

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• early symptoms are difficult to discern, appearing as small yellow spots (1-10cm in diameter) • 'spots' consist of dense clusters of excessively tillered yellow-white shoots, with few roots arising from a single bud in the crown and stolons • under humid conditions, white mycelia develop on leaf surfaces • yellowing of infested patches occurs during cool, wet weather, while withering and browning occurs with high temperatures, drought or winter stress • the basal tillers are unscathed, resulting in disease reoccurrence the following season, with different levels of severity • persistence of favourable disease conditions results in severe stunting • on individual leaf blades, white linear streaks, running parallel to the midvein appear; leaves are chlorotic with tip necrosis • leaf blades may appear broader and thicker than healthy blades	• disease is prevalent from late winter to early autumn • disease occurrence is noted under cool (10-25°C), wet conditions and high humidity • water-saturated soils or low lying areas are prone to outbreaks	• disease reoccurrence is seen to spread outwards from the initial infection site • pathogen overwinters as dormant oospores or mycelia in living plant material, thatch and plant debris • under favourable conditions (15°C.) the fungus sporulates, producing many sporangia. Sporulation is abundant on *Digitaria* spp., which is an alternate host to further infections • in the presence of free water the sporangia release motile zoospores, which encyst • these germinate producing a germ tube, which forms an appressorium • plant penetration is direct, leading to intercellular infection • sporulation and dissemination occurs 4-6 hours thereafter	• *Stenotaphrum secundatum* is most commonly and severely affected • other cool-season grasses infected are: *Poa* spp.; *Festuca* spp. and *Lolium* spp. • Gramineous hosts, which includes *Saccharum* spp., exist and act as alternate hosts	***Cultural practices:*** at present cultural control is achieved by preparing seedbeds, with adequate drainage to remove excess surface water • core to improve soil infiltration rates and to prevent soil compaction and surface crusting • wet grass should never be mowed • management should ensure that active growth is maintained ***Chemical control:*** • fungicides registered for *Pythium*-related diseases are effective • fungicides with the following active ingredients are recommended: copper ammonium carbonate, copper hydroxide, copper oxychloride, copper oxychloride/sulphur; cupric hydroxide, folpet/sulphur, mancozeb, metalaxyl, propmocarb hydrochloride and zineb	• destructive disease of cool-season pastures and turfgrasses, with outbreaks reported in Australia, Canada, Europe and the U.S.	Jackson, 1980; Turgeon, 1991; Smiley *et al.*, 1992; Couch, 1995; Emmons, 1995; Krause *et al.*, 1996; Nel *et al.*, 1999

[g] Downy mildew is also referred to as yellow tuft or simply yellows

Table 2.5 Summary table of *Claviceps purpurea* (Fr.:Fr.)Tul., commonly referred to as ERGOT

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• disease affects seedheads • infection includes the formation of a sticky, syrup-like substance from the floret and the formation of small black sclerotia, which replace the seed in the inflorescences • disease is initiated at the 'honeydew' stage. Closer examination of the developing florets reveals ovaries that are a white, slimy mass • eventually this develops into horn-shaped, purple-black sclerotia which protrude beyond the floral bracts . Other saprophytic pathogens may also be present • floral tissues mature before sclerotia are able to form and the disease is unable to develop beyond the honeydew stage	• disease occurrence is noted with the onset of wet weather and the opening of the florets • disease is prevalent under cool, damp weather associated with the onset of spring	• disease is recognised by the production of a sticky 'honeydew' consisting of conidia • insects feed on the 'honeydew', dispersing the conidia • conidia may also be dispersed by means of water splash • mature sclerotia fall from the floret to the ground or are mixed with the seed at harvest, aiding long-distance dispersal • sclerotia serve as overwintering structures which germinate to produce stromata and perithecia in spring • ascospores are discharged from fruiting structures and are caught by air currents and deposited on developing florets	• disease is common to *Lolium* spp. and *Pennisetum glaucum* (L.). R. Br. and other cultivated grass spp. • pasture and turf cultivars commonly affected are: *Agrostis* spp.; *Bromus* spp.; *Festuca* spp. and *Poa* spp. • physiological specialization of the causal agent restricts infections	***Cultural practices:*** primarily include deep ploughing and crop rotations • eliminate weeds by mowing and grazing before flowering, or at the earliest stages of flowering • burn the infected grass stand • at planting ensure that only certified, disease-free seed is used • sclerotia can be removed from contaminated seed by soaking the seeds for 3 hrs in water and then 'floating-off' the sclerotia using a solution of 18kg salt/100ℓ of water ***Chemical control:*** at pre-anthesis offers more control by reducing the primary inoculum • chemical control involves fungicide and herbicide usage; herbicides are used to eliminate alternate hosts • a single foliar application of fungicides with the active ingredients: flusilazole, propiconazole and tebuconazol reduce sclerotia and exudate to zero, or near to zero	• important disease in the grass seed producing industry, attacking seedheads of grasses and cereals world-wide. • yield losses are primarily through seed replacement, reduced seed production, reduced seed weight, lower seed vigour and loss of healthy seed when cleaning to remove ergot • under good management of turfgrasses the disease is of little importance, as seedheads rarely form. Disease does, however, impact indirectly on seed availability • Ergot is poisonous to humans and livestock when ingested • it poses a major problem on pastures, especially spring planted ryegrass as it is responsible for livestock abortions	McVickar and McVickar, 1963; Moore, 1966; Hall, 1992; Smiley *et al.*, 1992; Schultz *et al.*, 1993; Lenné, 1994b; Mohamed-Saleem and Berhe, 1994; Couch, 1995; Alderman *et al.*,1998

Although all fairy rings appear to be similar, there are two known types of rings. The characteristics of each type and the similarities that exist between the two have been summarised in Tables 2.6.1 and 2.6.2.

Table 2.6.1 Summary table of EDAPHIC FAIRY RINGS

Causal agent	Symptoms[h]	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• soil-inhabiting fungi • 54 causal agents belonging to the subdivision Basidiomycetes have been identified	• initally small circles or half-circles of dark green (due to N release), faster growing grass; rings expand concentrically as disease progresses reaching 0.5 -10 m • rings may be off-set by thinning dead grass just within or outside the rings • in severe cases, fungal growth occurs within the ring • soil becomes hydrophobic, resulting in thinning, killing the grass (dry spots) • rings may coalesce, forming scalloped edges due to fungal growth ceasing at contact points • rings are classified according to symptom expression: Type I - damage or kill the grass; Type II - stimulates growth Type III - no apparent influence on growth • fungal fruiting bodies become apparent, arranged in orderly periphery rings of an inner zone of dead grass and an inner circle of stimulated grass growth	• rate of spread and diameter of the ring is predetermined by prevailing weather conditions and fungal species present • heavy mushroom infestations are associated with the use of organic fertilizers; high N fertility increasing edaphic fairy ring intensities	• initiated with the translocation of mycelial inoculum, or from the germination of basidiospores • the initial fairy ring (following primary infection) is seen to have a small cluster of fruiting bodies from which mycelia penetrate into the surrounding area actively colonizing the soil and grass • rate of spread is limited by areas void of vegetation or areas already colonized by the causal agents • fungicides and soil sterilisation may increase fairy rings by altering thatch and soil microorganism populations	• all cultivated grass species are prone to infection • small grains are also affected	• most effective means of control is by fumigation and excavation; this is not economically viable or always successful ***Cultural control:*** aimed at correcting conditions that predispose the area to fairy rings • strip the sod from the infected area; follow with soil fumigation; replant with disease-free sods or seed • avoid 'spilling' infested soil onto adjacent, healthy grass potentially initiating new rings • mixing non-infected and infected soil reduces incidences • improve irrigation techniques to prolong water saturation . Irrigate every second day, to a depth of 30cm, maintaining this for 4-6 weeks; or inject water into the rings or dig and fill deep holes in the soil profile with water. Increased soil moisture overcomes hydrophobic soils and increases antagonism • avoid thatch accumulation, by vertical cutting and coring ***Chemical control:*** is erratic as the fungal pathogen is often present deep in the soil • fungicide applications must therefore not only be to the foliage but also to the subsurface soil, as a soil drench. • fungicides available include: flutolanil and methyl bromide	• decreases the aesthetic value and playing quality of turf surfaces • mushrooms may be poisonous, having serious consequences to humans and animals	Shantz and Piemeisel, 1917; Turgeon, 1991; Smiley *et al.*, 1992; Couch, 1995

Table 2.6.2 Summary table of LECTOPHILIC FAIRY RINGS

Causal agent	Symptoms[h]	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• leaf litter or thatch inhibiting fungi • causal agents have been identified as *Coprinus kubickae* Pilat and Sverck, *Marasmius siccus* (Schwein) Fr. and *Trechispora alnicola* (Bourd. and Galz.) Liberta. • may also be initiated by sterile Basidiomycetes	• common to bowling greens and golf greens • initially appears as small circles or half-circles of fruiting bodies (usually mushrooms) or darker green grass; yellow grass or dead grass; rings expand concentrically as disease progresses reaching diameters of 0.3-1.8m • a white mycelium may be observed on the lower leaves of the infected plants or in the underlying thatch layer • soil and thatch becomes hydrophobic, resulting in dry spots • rings may coalesce, forming scalloped edges due to fungal growth ceasing at contact points • rings are classified according to symptom expression: Type A - may or may not produce mushrooms; sparse to abundant mycelia develop on the base of shoots or in thatch, with little or no effect on growth Type B - stimulates growth and/or discolouration; plants are not severely injured by infection and recover; thatch degradation is apparent Type C - in the initial stages may or may not stimulate growth; eventually the grass is severely injured	similar to Table 6.1 with addition of: • factors stimulating the formation of a thick thatch layer have a direct effect on initiation	similar to Table 6.1 with addition of: • *T. alnicola* is pathogenic to grass roots, with direct penetration of the root tissues • several sterile Basidiomycetes have shown pathogenicity to lower stems, crowns and roots of grasses; colonization is inter- and intra-cellular	similar to Table 2.6.1	similar to Table 2.6.1	similar to Table 2.6.1	Smith *et al.*, 1989; Turgeon, 1991; Couch, 1995

[h] Rings experience disrupted growth and may disappear without warning

There are a number of *Fusarium* spp. that are pathogenic to grasses. Fusarium diseases have been included due to their effect on seedling establishment and as a comparison to Pythium and Rhizoctonia damping-off.

Table 2.7.1 Summary table of *Fusarium culmorum* (*Microdochium nivale* (Fr.) Samuels and Hallett)[1], commonly referred to as FUSARIUM DAMPING-OFF[2]

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• postemergent diseases associated with young seedlings (two to three weeks after emergence) • infection in the stand is characterised by small, light yellow, turning brown, scattered patches • individual seedlings are light yellow, turning a bronzy-brown colour • the plant is unable to support upright growth and the plants collapse, as the stem withers • also infects ungerminated seeds, resulting in a low germination percentage due to the seed rotting	• *F. culmorum* results in disease in the summer (warm weather patch disease) • *M. nivale* results in disease in the spring (winter patch disease) • disease symptoms are more apparent in the summer when the average air temperatures range from 10-20°C and in the spring when the average temperature reaches 20°C • symptoms are commonly noted in dry soils	• causal agents colonize thatch and soil, initiating disease when temperature requirements occur • is also transmitted/spread by seeds • disease incidence is therefore high when areas have been previously infected and are reseeded / overseeded	• common to all plants	***Resistance:*** must be combined with seed treatments for efficiency valuable in the control of damping-off • high resistance levels are expressed by: *Lolium perenne* and *L. multiflorum* • moderate resistance to susceptibility expressed by: *Dactylis glomerata*, *Festuca arundinaceae*, *F. pratensis* and *Pheum pratenses* ***Cultural practices:*** sow only fresh seed, with high germination and establishment rates, avoiding high seeding rates and stand densities • avoid planting seeds into warm, dry soil which are prone to infection and colonization; rather ensure adequate moisture before and during seedling germination and emergence • ensure the correct planting date and an even nutrient reserve from germination to maturity • sustain a balance between N, P and K ***Biological control:*** • in the U. S. there are 8 registered antagonists. These include: fungi - *Gliocladium virens* G-21 and *Trichoderma harzianum* KRL-AG2; bacteria - *Agrobacterium radiobacter* K84, *Pseudomonas fluorescens* EG1053, *Berkholderia cepacia* type Wisconsin, *Bacillus subtilis* GB03, *B. subtilis* MBI 600 and *Streptomyces griseoviridis* K61 ***Chemical control:*** involves seed treatments prior to planting • effective fungicide active ingredients include: thiram, benomyl, captan, fosetyl-Al or furalaxyl • fungicide mixtures of benomyl and captan provide excellent control on susceptible cultivars • when reseeding/overseeding a previously infected area a preplanting fungicide application is essential, with reapplications at emergence, and a strict preventative spray program	• disease is commonly encountered, especially on seedlings • known to cause severe losses in establishment of pastures and turf stands	Holmes, 1983; Lewis, 1985; Couch, 1995; Krause *et al.*, 1996; Kim *et al.*, 1997; Nel *et al.*, 1999

[1] There are two known causal agents

[2] Damping-off is a term that is commonly associated with seedling diseases, whether it be as a result of a physiological disorder or a pathogenic agent

Table 2.7.2 Summary table of *Microdochium nivale*[k], inciting FUSARIUM PATCH[l]

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• in the absence of snow, disease initially appears as small, orange-brown, water-soaked, spots, 5-7.5 cm in diameter • within 48-72 hrs spots coalesce into large circular brown patches, 20-30cm in diameter • diseased areas are reddish-brown turning tan becoming blighted as shoots die • close examination of the patches reveals matting infected plants • a white or faint-pinkish mycelial border (depending on grass species) appears in the early mornings and under high humidity • a 'frogeye' pattern may develop, where the centre of patches recover resuming healthy green growth • in the presence of snow, circular patches appear as the snow melts • these are dull white, now measuring 7.5-30cm in diameter • patches closest to the snow line are covered by a white to pale pink cottony mycelium, which mats the grass leaves • extended leaf wetness results in numerous pink coloured sporodochia developing on the leaf surfaces, hence the name pink snow mould • weed and moss invasions are common	• disease occurrence is associated with high humidity, wet conditions and air temperatures >15°C • extended leaf wetness, even at 18°C results in disease • temperatures of 21°C or higher render *M. nivale* non- pathogenic • a thick snow cover on an unfrozen ground is also conducive; plants are unable to photosynthesise, predisposing plants to infection • snow cover is not a requirement, as symptoms occur regardless	• pathogen over-winters as dormant mycelia in living or dead plant material • when conditions improve, conidia are produced and are dispersed by means of water splash • penetration is via natural plant openings and wounded plant tissue • growth and colonization is intercellular • conditions that predispose plants to disease are frost, mechanical wounding, soil pH of 6.5 or higher and N applications in the growing season or during winter	• cool season grasses are prone to disease • this includes: *Agrostis tenuis*, *A. palustris*, *A. canina*; *Cynodon dactylon*; *Poa annua*, *P. pratensis*; *Lolium multiflorum*, *L. perenne*; *Festuca rubra* var. *commutata*, *F. ovina*, *F. arundinacea* and *F. rubra*	***Resistance:*** *P. annua* and *Agrostis* spp. are most susceptible; *P. pratensis*, *L. perenne* and *Festuca* spp. are moderately resistant becoming blighted but with recovery • if disease is common plant the more resistant fescues and bentgrasses ***Cultural practices:*** based on keeping the turf surface dry • remove dew in the morning by brushing the grass with a fibreglass pole • prevent the accumulation of snow on the grass surface • maintain thatch at 1.3 cm or less, to maintain adequate moisture levels • maintain low pH and suitable soil fertility to reduce disease severity; apply fertilizers, especially N, early in winter to prevent lush late season growth • limit use of infected pastures, allowing short intervals of grazing in autumn/winter; do not allow the stand to grow out ***Chemical control:*** should be combined with fertilizer applications and an extended grazing regime • fungicide resistance is high and should be integrated with other control measures or rotated in a fungicide program • commence applications with disease onset or when conditions suitable for disease development; applications should continue while cold, wet weather persists • it is not recommended to spray preventatively or to spray a well established infection, except when using dicarboximides • fungicides with the following active ingredients are recommended: mercury chloride (banned in S.A.), triadimefon, cyproconazole, propiconazole, thiophanate methyl, benomyl or fenarimol • benlate, applied in late autumn; carbendazim and thiabendazole in the spring/summer and dicarboximide fungicides (iprodione and vinclozolin) applied in the winter	• commonly associated with cool season turf and pasture grasses • disease occurrence is favoured by cold, wet, humid conditions associated with late autumn or early spring • severe outbreaks occur under snow cover, but severe outbreaks are also associated with the absence of snow cover as commonly observed in S.A.	Vengris and Torello, 1982; Baldwin, 1990; Turgeon, 1991; Smiley *et al.*, 1992; Couch, 1995; Emmons, 1995; Schroeder and Sprague, 1996; Mercer and Ruddock, 1997

[k] This causal agent is also responsible for inciting Fusarium damping-off

[l] Fusarium patch is also commonly known as pink snow mould

Table 2.8 Summary table of *Drechslera* spp., *Bipolaris* spp., *Exserohilum* spp., inciting HELMINTHOSPORIUM LEAF SPOT[m]

Causal agent	Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• the causal agents are facultative saprophytes and are classified under Fungi Imperfecti • *Drechslera* spp. produce brown mycelia which grow in the host or along the host surface, giving rise to conidiophores and conidia; the conidial sizes and shapes are characteristic for each *Drechslera* spp; germ tubes are produced perpendicular to the conidium axis • *Bipolaris* and *Exserohilum* spp. closely resemble the *Drechslera* spp. except that conidia gradually taper at both ends (fusoid), they are also curved rather than straight; conidia produce germ tubes only from the ends of the cells	• in general, symptoms include reddish-brown to purplish-black lesions on the grass leaves, measuring 1-4mm • leaf spots are more concentrated near the collar of the leaf blade • in severe cases the crowns, stems and rhizomes are also infected, becoming discoloured and chlorotic, with wilted leaves and eventually the infected plant dies • symptoms are variable differing for each causal agent	• occurrence is noted under cool, moist conditions and overcast days, usually in spring and autumn; however, exceptions occur • pathogenicity is limited only by frozen soil or very hot weather • conidia are generally present at temperatures ranging from 3-27°C and extended periods of high humidity • N fertilization influences plant susceptibility	• pathogen overwinters as conidia or dormant mycelia in infected plant tissues and debris • when conditions improve, with extended leaf wetness and moist leaf litter, sporulation occurs forming conidia • spore dispersal is by wind, irrigation water (in the late afternoon), equipment (especially mowers), traffic, infected seed and leaf fragments • hypothesised that disease increases as the leaf sugar content is reduced • postemergent herbicides 2,4-D, MCPP and dicamba increases sporulation and hyphal growth of *B. sorokiniana*, increasing severity of leaf spots	• common to cool- and warm-season grasses	***Resistance:*** variable amongst grass species • resistant varieties should be chosen for infected areas, although susceptibility to other diseases is expressed ***Cultural control:*** commonly cited methods involve raising the mowing height, avoiding excessive nitrogen fertilization, improving irrigation scheduling, controlled use of herbicides and fungicides, and improving drainage • in pastures implement an extended grazing regime, together with fungicide applications • in contradiction the reduction of traffic will reduce plant stress , compaction and reduce spread • thatch buildup increases moisture and is reduced by topdressing, removing grass clippings and ensure that grazing is continuous ***Biological control:*** *Stenotrophomonas maltophilia* strain C3 has been evaluated for the control of *B. sorokiniana* (melting-out) and proven effective, but is not yet registered ***Chemical control:*** fungicides are effective against the leaf lesion stage of the disease • recommended fungicide active ingredients include: anilazine, chlorothalonil, copper ammonium carbonate, copper hydroxide, copper oxychloride, copper oxychloride/sulphur, iprodione, folpet/sulphur, mancozeb, maneb, tebuconazole, triadimefon and zineb	• disease is of little threat to pasture grasses; yield is not affected; may affect quality of the pasture in terms of carbohydrate and N content or digestibility • grasses are still grazed although disease intensity increases until conditions become unfavourabe for further development • in general, although disease incidence may be high, disease intensity is usually low • in turfgrasses, disease occurrence detracts from the aesthetic appeal and control measures must be implemented	Cole *et al.*, 1969; Vengris and Torello, 1982; Lam and Lewis, 1983; Hall, 1992; Smiley *et al.*, 1992; Dernoeden, 1993; Hodges, 1994; Lenné, 1994b; Couch 1995; Emmons, 1995; Krause et al., 1996; Schroeder and Sprague, 1996; Mercer and Ruddock, 1997; Nel *et al.*, 1999; Zhang and Yuen, 1999

[m] *Drechslera* spp. incite diseases such as kikuyu and cynodon leaf spot; melting out; Helminthosporium blight; Drechslera leaf blight; brown blight; leaf blotch; zonate eyespot; Helminthosporium root, crown and rhizome rots; stem and crown necrosis; and net blotch, while *Bipolaris* and *Exserohilum* spp. are commonly responsible for Helminthosporium leaf, crown and root diseases.

Table 2.9 Summary table of *Verrucalvus flavofaciens* Wong & Dick, the proposed causal agent for KIKUYU YELLOWS[n]

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• symptoms appear in late spring when plants are under moisture and nutrient stress • symptom expression is minimised by adequate soil fertility and moisture • the causal agent invades the grass roots, becoming systemic in the stems and leaves, which turn bright yellow and eventually die • yellowing occurs in scattered, irregular patches, which increase in diameter; the centre of the patches slowly decline in productivity and also die; often centres of the patches are invaded with weeds and C_4 grasses • further symptoms include: shortened internodes and a weakened root system, infected plants are easily pulled from the soil, revealing rotted, yellow brown roots • kikuyu yellow symptoms are often associated with *Bipolaris cynodontis.*, and therefore is often overlooked as being a leaf spot disease • on pastures there is no difference in percentage digestible carbohydrates, crude fibre and digestible nutrients between healthy and infected kikuyu, but a reduction in crude protein occurs	• disease occurrence is noted in late spring (November) and throughout the summer • outbreaks are commonly associated with hot, humid conditions • fungal growth is abundant above a minimum temperature of 15°C, disappearing with the onset of cooler temperatures	• infection is via the root system • new infections are incited by the germination of aplanospores, produced in asexual reproduction, or via the oospores, produced in sexual reproduction	• disease common only to *Pennisetum clandestinum*	• no method provides complete control of this disease ***Resistance:*** is available; Noonan is a tolerant cultivar • resistance breeding through mutagenesis is ongoing ***Cultural practices:*** are numerous • apply phosphate and lime to correct Molybdenum (Mo) deficiencies commonly associated with acidic soils • when the minimum temperature falls below 15°C apply N fertilizer to the infected area to stimulate root growth • remove infected grass, by means of grazing or mulching ***Chemical control:*** control measures are numerous • biofumigation is at present under investigation: the incorporation of mustard seed meal into soil and the decomposition of *Brassica* plants releases isothiocyanate which 'fumigates' soil • fungicides with systemic activity against oomycetes are recommended • herbicides, such as glyphosphate, can be applied to the infected area including a radius of 50cm of healthy kikuyu, to kill the infected area, which can be resown after a fallow year	• interest in this disease is growing, as new outbreaks occur • yellowing and thinning detracts from the aesthetic and playing value of a turf stand • in pastures, disease decreases productivity but has no apparent influence on the palatability; however, many farmers are cautious about grazing the infected pasture	Wong, 1975; Dick *et al.*, 1984; Tesoriero, 1990; Hall, 1991; Turgeon, 1991; McKirdy and Jones, 1993; Lenné, 1994b; Wong and Fulkerson, 1996;

[n] It has been suggested that the causal agent may be either viral or fungal, or that it may be due to Molybedum (Mo) deficiencies which induces yellowing

There has been little research done on Kikuyu yellows in S. A., although it is becoming a prevalent disease. Pathologists in Australia and N.Z. are responsible for the research and information available on this disease.

Table 2.10 Summary table of *Pythium* spp.°, inciting PYTHIUM DAMPING-OFF

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• stand establishment appears patchy, with areas of 2.5 -31cm in diameter where seedlings are either dead or absent • as a pre-emergent disease infection occurs when the seed coat splits, and seedling emergence is absent • as a post-emergent disease, seedling stems appears necrotic at the soil surface; as disease progresses the entire stem becomes necrotic and water-soaked and the stem collapses • under humid conditions, a white mycelium is seen on the collapsed mass of stem tissue	• the causal agents are present within the soil as oospores • for infection and colonisation of the seedlings or seed, air temperature should be between 13-28°C	• oospores form the resting bodies responsible for initiating new infections • when conditions improve these germinate producing mycelia which are responsible for infection and colonisation • long distance dispersal is by means of soil containing oospores or by means of run-off water over the soil surface and traffic	• common to all grass species	• for a general outline on the control of this disease refer to the control of Fusarium damping-off in Table 2.7.1 • damping-off control measures are identical, except for a few changes specific to the pathogen type ***Cultural control:*** involves the use of suppressive composts ***Biological control:*** has great potential with two biocontrol agents identified; *Bacillus* L324-92 and *Gliocladium virens* GL-21 ***Chemical control:*** fungicides proves effective. Metalaxyl applied as a seed treatment provides control and should be reapplied at seedling emergence and then followed by a strict preventative spray program, such as previcur	• Pythium damping-off poses a serious problem in the establishment and overseeding of pastures and turf • disease is most severe on young seedlings, but will also attack grass at any stage of development, killing the grass in only 24 hrs	Turgeon, 1991; Couch, 1995; Craft and Nelson, 1996; Kim *et al.*, 1997; Koch, 1999

° There are a number of *Pythium* spp. pathogenic to grass seedlings. These pathogenic *Pythium* spp. are also responsible for Pythium blight

There are a number of *Pythium* spp. that are pathogenic to grasses. Pythium damping-off causing severe damage in establishing grass stands.

Rhizoctonia diseases are considered to be economically important soil-borne diseases. There are two common *Rhizoctonia*-incited diseases that are associated with grass species; being Rhizoctonia damping-off and Rhizoctonia blight.

Table 2.11.1 Summary table of *Rhizoctonia solani* Kuhn, inciting RHIZOCTONIA DAMPING-OFF

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• predominately a post-emergent disease, but also causes pre-emergent seedling death • initial symptoms are discolouration of the stem at the soil surface, with a dry rot developing up the stem • the stem withers, with a "wire-like" appearance and collapses • the entire plant shrivels, turns light-brown in colour and dies	• disease occurrence is noted when the optimum temperature ranges between 16-24°C	• pathogen overwinters as sclerotia in soil/thatch • when conditions improve sclerotia germinate producing mycelia, initiating primary infections • sclerotia are also transmitted by seed, accounting for long distance dispersal	• common to all grass species	• for a general outline on the control of this disease refer to the control of Fusarium damping-off in Table 2.7.1 • damping-off control measures are identical, except for a few changes specific to the pathogen type ***Biological control:*** has great potential with three biocontrol agents identified; *Bacillus* L324-92, *Gliocladium virens* GL-21 and *Bacillus subtilis*, applied as a slurry coat formulation ***Chemical control:*** includes seed-applied fungicides • fungicide active ingredients include: carboxin, iprodione and pentachloronitrobenzene; resistant or tolerant strains do exist	• disease poses a serious problem in the establishment and overseeding of pastures and turf • on turf, damping-off is a problem in the overseeding of dormant warm-season with cool-season varieties in order to maintain the aesthetic value of a stand during winter	Couch, 1995; Fiddaman and Rossall, 1995; Cubeta and Vilgalys, 1997; Kim *et al.*, 1997; Koch, 1999

Table 2.11.2 Summary table of *Rhizoctonia solani* Kuhn, inciting RHIZOCTONIA BLIGHT[p]

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• symptoms are variable according to the grass species, height and prevailing conditions • circular patches appear, 5-50cm in diameter in short and 30cm-1m in taller grass; patches are thinned and blighted with a tan-brown colour • the patch centre may recover, giving a "frogeye" appearance • the patches may coalesce to form large irregularly shaped areas of blighted grass • when humidity is high and temperatures warm, patches have a "smokey, fungal mycelial ring" in the early morning; this forms on the outer edge of a water-soaked, bluish-purplish centre. This is common to severe occurrences • as the day progresses, patches become a uniform light brown colour • light infections only kill the leaves and the grass recovers quickly • heavy infections, kill the stems and crowns with extensive soft rot occurring at the base of the leaf sheath and stems and the plants are easily pulled from the soil; there is no recovery rate • individual leaf lesions are initially small and tan, enlarging under warm, humid conditions, turning red-brown • lesions coalesce, and entire leaves becomes necrotic, dry and brittle • leaf characteristically maintains shape	• on cool-season grasses disease occurrence is noted in the summer months; with the onset of warm to hot, humid, moist conditions • an average temperature of 18-20°C results in the germination of sclerotia • infection occurs with 48hrs of leaf wetness, temperatures from 23-30°C and a humidity of 90-100% • colonization is rapid when temperatures are 29-32°C. • disease development is also influenced by: high planting densities, leaf wetness, soil fertility (especially N), soil pH and drainage • cool season Rhizoctonia blight also occurs, affecting warm-season grasses in spring and autumn	• pathogen overwinters as dormant mycelia or as sclerotia in plant debris; *Rhizoctonia* spp. also grow and survive saprophytically in soil • sclerotia are disseminated via wind, rain and animals • when the temperature is 18-20°C, mycelia emerge from the sclerotia; at 23°C initial infection occurs, with temperatures of 23-29°C being associated with penetration; at 32°C pathogenicity is lost • tissue colonization is both inter- and intra-cellularly and is due to enzymes and toxin formation	• epidemiology and symptoms vary with grass species • all grasses are infected; *Agrostis* spp., *L. perenne*, *S. secundatum* and *Poa annua*, being the most seriously affected of these	***Resistance:*** is variable amongst the grass cultivars ***Cultural control:*** involves improving management practices • avoid excess N use and/or applications in late summer or autumn • use a slow soil acidifying source, such as sodium nitrate • P and K should be combined with N applications for recovery • ensure optimum soil pH, correcting acid pH levels • reduce excess water due to poor surface and subsurface drainage, incorrect irrigation, and remove early morning dew where possible by means of raking or dragging a water hose across the grass surface; do not plant highly susceptible cultivars in high rainfall areas • irrigate at night or in the mornings to reduce leaf wetness by 2-4 hrs • avoid mowing in the early morning when dew is present; remove clippings promptly • ensure the correct mowing height; avoiding low heights • burn infected pasture (based on the pasture's fire-tolerance) • continuous grazing will eventually reduce disease • scout for early disease symptoms ***Biological control:*** potential has been explored with three fungal antagonists identified: *Gliocaldium* spp., *Penicillium* spp. and *Trichoderma* spp.; and a number of bacteria (including *Bacillus subtilis*) • biocontrol activity is however insufficient on very susceptible hosts ***Chemical control:*** with a fungicide program is essential, especially once the night temperature is >21°C and humidity is >85% • control is optimal when fungicides are integrated with cultural practices • recommended fungicide active ingredients include: anilazine, benodanil, chlorothalonil, cyproconazole, iprodione, mancozeb, propiconazole and triadimefon • applications should be as a soil drench at the first signs of infection • on pastures chemical control is costly and the possibilities of chemical residues in milk and meat may pose a health risk to humans	• one of the more important diseases of turfgrasses in the U.S. • reports of severely infecting pasture grasses are limited, although on tropical pastures it has been reported to cause production losses of 20-100% dry matter • disease is most severe under hot, humid weather conditions resulting in large disease areas, with no recovery rate	Shurtleff, 1953; Bloom and Couch, 1960; Vengris and Torello, 1982; Howard, 1986; Turgeon, 1991; Burpee, 1992; Smiley *et al.*, 1992; Davis and Grof, 1994; Davis and Irwin, 1994; Lenné, 1994 b; Trutmann, 1994 a & b; Couch, 1995; Emmons, 1995; Fiddaman and Rossall, 1995; Fidanza and Dernoeden, 1996; Giesler *et al.*, 1996; Schroeder and Sprague, 1996; Cubeta and Vilgalys, 1997; Laing, 1997; Fraser *et al.*, 1999 b & c

[p] Rhizoctonia blight is also referred to as large brown patch or more specifically brown patch

Table 2.12 Summary table of *Puccinia* spp.; *Uromyces*, spp.; *Uredo* spp. and *Physopella* spp., responsible for inciting RUST

Causal agent	Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• 13 reported species causing rust • species are all obligate parasites • differentiated according to their teliospore and uredinial characteristics	• different pathogenic species are responsible for symptoms on the leaves, stems and crowns • in general, first symptoms are seen as light yellow spots almost covering the entire upper leaf surface; and later in the season the lower leaf surface too • these spots soon become orangish-red/brown (rusty) pustules and the fruiting bodies burst through the cuticle • the pustules produce black teliospores which are the overwintering structures • as the disease progresses, leaves turn from yellow to brown; the stand becomes severely thinned • infection is short-lived and rarely kills the grass	• disease occurrence is associated with a wide range of temperatures, and humid conditions. Symptom expression is most prominent in autumn and spring • infection is determined by light intensity, temperature and humidity; for initial infections 4 to 8 hrs of low light intensity, temperatures of 21-24°C and high humidity are required • tissue penetration requires 8 to 16 hrs of high light intensity, temperatures of 30-35°C and a reduction in leaf surface moisture • low N fertilization, with poor soil moisture, contribute to infection rates	• the pathogen overwinters as dormant mycelial fragments or as teliospores • in early spring the teliospores germinate, giving rise to basidiospores; these are wind dispersed, initiating infection on alternate hosts, giving rise to pycniospores • the next spore stage is aeciospores; these are wind dispersed over short distances, infecting suitable hosts producing urediospores • urediospores are wind dispersed, infecting grass hosts • in autumn teleospores are formed and the cycle begins again	• disease is common to both cool- and warm-season grasses • alternate hosts are commonly herbaceous and woody plants	***Resistance:*** is available; with susceptible and resistant cultivars planted together as mixtures, for effective control • resistance combined with improved managerial practices offers the best means of disease control ; however, regular disease monitoring is required as resistance may be easily matched • resistant breeding programmes are ongoing ***Cultural control:*** involves not planting susceptible grass cultivars into infertile soils, into shaded areas or when drought conditions persist • avoid growth-limiting conditions; ensure that N fertilization is adequate and that field capacity is maintained through irrigation • schedule night irrigation to reduce the duration of leaf wetness • remove grass clippings after mowing • on pastures, allow the area to grow out and then grazing the area ***Chemical control:*** • registered fungicide active ingredients include: chlorothalonil, copper ammonium carbonate, benodanil, copper hydroxide, copper oxychloride, cupric hydroxide, mancozeb, oxycarboxin, triforine and zineb	• rust seldom kills the host plant, but if it occurs simultaneously with other foliar pathogens it may cause severe damage • on turfgrasses the occurrence of disease detracts from the aesthetic and playing quality of the stand • on pastures, productivity is reduced due to severe leaf losses; discolouration of the grass and the dusty residue, decreases palatability • in general poorly managed stands are most prone to disease	Heimes and Loecher, 1980; Vengris and Torello, 1982; Turgeon, 1991; Hall, 1992; Smiley *et al.*, 1992; Dernoeden, 1993; Welty and Barker, 1993; Lenné, 1994b; Couch, 1995; Emmons, 1995; Krause *et al.*, 1996; Schroeder and Sprague, 1996; Laing, 1997; Agrios, 1997; Roderick and Thomas, 1997; Nel *et al.*, 1999

Table 2.13 Summary table of *Leptosphaeria korrae* Walker and Smith; *L. narmari* Walker and Smith; *Gaeumannomyces graminis* (Sacc.) von Arx and Oliver var. *graminis* and *Ophiosphaerella herpotricha* (Fr.) Walker, responsible for inciting SPRING DEAD SPOT

Causal agent	Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• four ectotrophic root-rotting fungi are responsible for inciting disease • of these *O. herpotricha* is considered to be the primary causal agent	• symptoms become apparent in a well-established stand (2-3 yrs or older), in early spring when grass dormancy breaks and regrowth commences • if overseeded or weed populations are high, symptoms are difficult to identify • symptoms appear as depressed, circular patches of straw-coloured grass, 15cm -1m in diameter • patches coalesce forming large, discoloured, irregular-shaped areas, often mistaken for winter desiccation • regrowth of the infected patches may be absent, with weeds colonizing the area • some patches may have a "frog-eye" appearance, as the warm-season grasses become re-established • if summer weeds are controlled, disease patches may recovered by the end of summer; but regrowth is notably slower • diseased areas may remain greener later into the season, increasing susceptibility of plants to injury by low temperatures • closer examination, early in the season, reveals small black/brown lesions on the base of the culm, crown, roots and stolons • a dry rot develops on culm bases and crown buds • the roots and stolons appear black and rotted, with strands of brown-black hyphae on the surface of the rotted roots and stolons • plants are easily pulled from the patches • sclerotia develop on the base of leaf sheaths and the culms beneath the leaf sheath; these may be associated with a brown hyphal growth	• root and stolon colonization occur in late summer or early autumn, when temperatures are 21-24°C • optimum conditions for infection and colonization are, however, in winter once the plants have reached full dormancy and temperatures are 10-15°C or less • disease is favoured by: a thatch layer >2cm; low K fertility and high N applications at the end of the growing season	• sclerotia present in plant material or soil, serve as a means of disease dispersal over long distances • primary infections occur via mycelial growth over the roots and stolons • penetration of epidermal cells is via infection pegs produced from mycelia or infection cushions • colonization of internal tissues may be intra- or inter- cellular	• mostly warm-season grasses are infected • disease is of particular importance in *Cynodon dactylon* and *C. dactylon x C. transvaalensis* Burtt-Davy stands	***Resistance:*** • cultivars with increased winter hardiness and greater shoot densities are able to withstand or recover from infections • *C. transvaalensis* is considered to be resistant; while bermudagrass cultivars are highly susceptible ***Cultural control:*** often results in the successful elimination of disease • avoid excessive N fertilization during the growing season, rather use the minimum rate recommended; avoid late autumn N applications • K fertility levels within the soil should be maintained at an adequate level, to improve winter survival; heavy late autumn applications of K should be avoided • use ammonium-based fertilizer programmes, such as ammonium chloride and ammonium sulphate • maintain thatch at a level that does not exceed 2cm, by maintaining the maximum height permitted as per use • avoid conditions that restrict root development; minimise factors leading to compaction and frequently core disease-prone areas to improve soil aeration ***Chemical control:*** • registered fungicide active ingredients are: benomyl, fenarimol and propiconazole • disease suppression may not be complete, but disease severity is reduced and infected areas may show recovery	• disease incidences are important where winter temperatures are low enough to induce winter dormancy in warm-season grasses • on turfgrasses, diseased patches not only distract from the aesthetic value, but also interfere ball movement • disease occurrence and severity is thought to be influenced by previous management practices	Smith, 1971; Beard, 1973; Kozelnicky, 1974; McCarty, Lucas and DiPaola, 1992; Couch, 1995; Baird *et al.*, 1998

Table 2.14 Summary table of *Gaeumannomyces graminis* (Sacc.) Arx and Oliv. var. *graminis*; inciting TAKE-ALL PATCH

Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• symptoms become apparent in early spring and late autumn • the stand appears severely thinned or dead, with roughly circular, depressed, blighted patches that enlarge • adjacent patches coalesce forming large, irregular patches, 0.6m or more in diameter • at the advancing patch margins, the plants appear yellow/reddish-brown, later becoming brown, and in winter have a greyish tinge • the patch centres are prone to invasions by other grass species and/or broad-leaved weeds, resulting in a 'frog-eye' appearance • during dry, warm weather plants are easily pulled from the infected patches, and the roots appear to be brittle and dark-brown to black in colour • closer examination reveals a dark brown to black ectotrophic mycelia on the roots, crowns, stolons and rhizomes and discolouration of the vascular tissues • mats of dark-coloured mycelia are also visible on and between the basal leaf sheaths, in late autumn the perithecia become apparent in the crowns and stem bases • a decline in the disease symptoms becomes apparent after 4-5 yrs	• disease occurrence is associated with cool (10-19°C), wet conditions, common to early spring and late autumn • symptom expression is pronounced during cooler conditions, such as during late summer and early autumn; once the plants have been exposed to heat and moisture stress • disease outbreaks are common to new sand-content based turf • occurrence is also affected by factors such as: a high level of surface moisture, soil pH >6.5; and soil fertility, where P has a suppressive effect • high rates of urea (N) increase plant susceptibility	• the pathogen survives as a parasite or as a saprophyte • disease spread is localized as the mycelia grow from plant to plant, and not through the soil • long distance dispersal is by the movement of infected soil and plant material, airborne spores and windblown dust particles	• disease is common to a number of cool-season grasses • these include: *Poa* spp.(these act as volunteer hosts); *Agrostis* spp.; *Festuca* spp. and *Lolium perenne*	Control is difficult once the disease has occurred, control measures should therefore be preventative ***Resistance:*** variable with some isolates of *G. graminis* being more pathogenic on certain grass species than others • *Agrostis* spp. are considered to be high susceptible to the pathogen ***Cultural control:*** offers optimum control • take regular soil samples to determine N, P and K deficiencies, especially if soils are sandy • apply acidifying fertilizers, such as ammonium chloride, ammonium sulfate or iron sulphate to acidify the soil surface and improve colour; N fertilization in the ammonium form, lowers soil pH and increases Mg availability, promoting seedling vigour and an extensive root system • avoid the use of lime. In general the maintenance of soil pH below 6 should be achieved, ensure that topdressing soils and irrigation water are below pH6 • remove diseased patches, when disease affects only small areas; and resod or overseed with less susceptible cultivars • maintain thatch, avoiding the buildup of a thick layer ***Biological control:*** • *Bacillus* L324-92 and the rhizobacterium *Pseudomonas fluorescens* shown antagonism towards *G. graminins* on wheat. The potential to apply these to grass species therefore exists ***Chemical control:*** • the application of fenarimol fungicides and the application of preventative fungicides such as, phenyl mercuric acetate drenches (banned in S.A.), propiconazole, myclobutanil and triadimefon based fungicides, late in autumn or early winter, provide some control	• disease incidence is of great importance on cool-season turfgrasses; detracting from the aesthetic value and playing quality • the first disease outbreak was noted in the 1950's when lime, which encourages disease development, was applied to correct extremely acidic soils • the disease is considered difficult to control, requiring optimum management when conditions favour disease development	Fravel, 1988; Baldwin, 1990; Turgeon, 1991; McCarty *et al.*, 1992; Smiley *et al.*, 1992; Dernoeden, 1993; Jackson, 1993; Couch, 1995; Elliot, 1995; Thompson *et al.*, 1995; York, 1996; Hill *et al.*, 1999

Table 2.15 Summary table of *Phyllachora* spp.; inciting TAR SPOT[q]

Causal agents	Symptoms	Epidemiology	Source and spread	Hosts	Control	Disease significance	References
• the first two pathogens identified were: *Phyllachora graminis* (Pers.) Fckl. and *P. silvatica* Sacc. and Speg. • another three pathogens have been added, these are: *P. bulbosa* Parbery; *P. cynodontis* (Sacc.) Niessl.; *P. fuscens* Speg. • causal agents are identified according to the shape, colour and arrangement of asci and ascospores	• in the stand, diseased grass patches are yellow-green to yellow patches • closer examination of the upper and lower leaf blade, reveals small, sunken, glossy or crusted black spots which are distributed linearly • lesions may be surrounded by a halo of chlorotic leaf tissue • as the leaves senescence the area surrounding the lesions remains green for longer, creating a green island effect	• disease occurrence and spread is favoured by cool, wet weather	• mycelia invade the leaf mesophyll and colonization is observed as yellowing of the leaf tissue	• disease occurs on a number of cool- and warm season grasses worldwide • the most susceptible grasses are the *Festuca* spp. • other hosts include: *Agropyron* spp.; *Agrostis* spp.; *Asprella* sp.; *Brachyelytrum* sp.; *Brachypodium* spp.; *Bromus* spp.; *Calamagrostis* spp.; *Cinnia* sp.; *Cynodon* sp.; *Panicum* sp.; *Pappophorum* sp.; *Phleum* sp.; *Poa* spp.; *Uniola* sp.; *Zoysia* sp.	No control measures have been published due to the lack of disease severity	• although this disease is common worldwide, the disease severity is minimal • on turfgrasses, disease detracts from the aesthetic value of the stand; in severe incidences, plant vigour decreases due to the leaves becoming chlorotic and necrotic • pasture production and persistence may be reduced	Couch, 1962; Lenné, 1994b; Couch, 1995

[q] Tar spot is also referred to as black leaf spot

2.3 PLANT PARASITIC NEMATODES AND THEIR EFFECT ON TURF AND PASTURE GRASSES

Nematodes are responsible for increasing a plants susceptibility to secondary infections by fungal or bacterial pathogens.

Table 2.16 Summary table of PLANT PARASITIC NEMATODES

Symptoms	Source and spread	Hosts	Control	Disease significance	References
• conclusive symptoms include a decline in quality not attributed to fungi, bacteria, viruses or physiological disorders, or fertilizer and fungicide applications • the presence of nematodes is determined by soil samples from which nematodes are extracted and identified • general infestations include a discolouration of the grass, in streaks or oval shaped patches; exceptions to this include foliar nematodes, where the seeds in the grass inflorescence are replaced with a purplish gall, leaf gall nematodes which produce purplish-pink galls at the leaf base • closer examination of the roots may reveal nematode predation and generally root appearance may include one or more of the following: suppressed growth (i.e. an increase in length), swellings, red or brown lesions on the roots, excessive root branching, necrotic root tips and general rotting of the roots • due to root colonisation the infected plants loose vigour and are unable to adjust to stress caused by moisture shortages, low nutritional status, high temperatures or herbicide damage and the plants are predisposed to secondary infections	• nematodes are present in almost all soils • plant damage is noted when environmental conditions (high temperature and humidity and water availability) are favourable for active plant growth, but destruction is greatest in plants predisposed by stress • nematodes require high populations to induce a decline in plant vigour • nematode activity is greatest when temperatures and humidity are high	• common to all grass species	• there are few practical control measures available ***Resistance:*** is variable with many resistance levels between species, and between cultivars and varieties within these species ***Cultural control:*** root-feeding nematode infections are reduced by encouraging root growth and development; grass seed nematodes are reduced by preventing the formation of inflorescences • encourage soil porosity and adequate aeration by tillage and correct irrigation scheduling • apply organic amendments to increase fertility and soil water-holding capacity, to aid in tolerance to nematode attack and stimulate biocontrol • avoid ammonium N fertilization which encourages higher nematode populations than when N is derived from an organic source • solarise or "cook the soil" before planting to decrease populations • ensure adequate sanitation principles between known infected stand and non-infected stands • implement crop rotation based on the nematode population(s) identified ***Chemical control:*** fumigant and non-fumigant nematocides are available but are extremely toxic and not economically feasible • on turfgrasses, aesthetic quality is of utmost importance, and here the use of nematocides is easy, effective and economically feasible based on the returns of a well maintained turf • when using nematocides always follow the manufacturer's recommendations, aerate the stand before application and water throughly upon application; note that applications on or with seed at planting allow for the use of low dosages • available nematocides include: aldicarb; ethoprophos; fenamiphos and fensulfothion • if the nematode population is below the "damage threshold level" nematocide use is not recommended; this threshold value differs based according to the grass species and nematode type	• infections are most common in sandy soils • in turfgrasses, this will detract from the aesthetic value of the area and if the damage threshold level is exceeded nematocides should be applied. • in pastures, infestations are of little concern • plant parasitic nematodes have the ability to destroy established and establishing plants • often nematodes are considered to be a more serious pest than any of the insect pests • there is, however, little information regarding the economic impact of nematodes on turf and pasture grasses	Spaull, 1985; van Bezooijen, 1985; Derneoden, 1993; Davis *et al.*, 1994; Stanton, 1994; Couch, 1995; Krause *et al.*, 1996; Montealegre *et al.*, 1996;

2.4 CONCLUSIONS

A grassland, whether it is used for amenity purposes or for pastures, is an ecosystem involving the interaction of abiotic and biotic factors. The interactions between plants and microorganisms or plants and environmental factors may be either detrimental or beneficial to the quality of the plant product. In terms of turf and pasture production it is this high quality product that must be maintained.

When planting grass, the persistence, quality and rate of establishment are of primary importance, all of which are impacted upon by disease outbreaks. Disease occurrence and severity is not only determined by pathogen populations alone, but is also influenced by cultural practices that spread or create ideal conditions for pathogen dispersal and proliferation. When maintaining a grass system there are a number of important influential factors to take into consideration. Factors such as soil condition, quality of irrigation water and frequency thereof, overall nutritional status of the soil, persisting weather conditions, height and frequency of mowing or grazing, aeration of the soil and turf canopy are important considerations. All these factors impact on disease suppression and could easily be integrated into an overall disease management system, involving other factors such as the use of agrochemicals, resistant cultivars and even biological control.

Increasing the awareness of farmers and turf managers to the early detection of diseases, their impact on the production system and methods for control, will impact greatly on the quality of the stand. The aim of this literature review was, to provide a comprehensive review on turf and pasture diseases encountered in the KwaZulu Natal midlands, identifying the causal agents and common disease symptoms, creating awareness of the factors that influence the disease spread, as well as control measures that could be implemented.

2.5 REFERENCES

Agrios G.N., 1997. Plant pathology, 4th edition. Academic Press, California: United States of America. p. 300-303.

Alderman, S.C., D.D. Coats, F.J. Crowe and M.D. Butler. 1998. Occurrence and distribution of ergot and estimates of seed loss in Kentucky bluegrass grown for seed in central Oregon. Plant Disease **82:** 89-93.

Atilano, R.A. 1983. Susceptibility of St. Augustinegrass germ plasm to *Pyricularia grisea*. Plant Disease **67:** 782-783.

Baird, J.H., D.L. Martin, C.M. Taliaferro, M.E. Payton and N.A. Tisserat. 1998. Bermudagrass resistance to spring dead spot caused by *Ophiosphaerella herpotricha*. Plant Disease **82:** 771-774.

Baldwin, N.A. 1990. Fungal diseases of sport turf. Mycologist **4:**16-19.

Beard, J.B. 1973. Turfgrass: science and culture. Prentice-Hall, Toronto: Canada. p. 488-501.

Bloom, J.R. and H.B. Couch. 1960. Influence of environment on diseases of turfgrasses. Phytopathology **50:** 532-535.

Brede, A.D. 1991. Interaction of management factors on dollar spot disease severity in tall fescue turf. HortScience **26:** 1391-1392.

Burpee, L.L. and L.G. Goulty. 1986. Influence of foliar-applied nitrogen on the severity of dollar spot. The Greenmaster **22:** 19. In: H.B. Couch (ed.). 1995. Diseases of turfgrasses, 3rd edition. Krieger Publishing, Florida: United States of America. p. 65-69.

Burpee, L.L. 1992. Assessment of resistance to *Rhizoctonia solani* in tall fescue. Plant Disease **76:** 1065-1068.

Cole, H., J. Duich, B. Taylor and G. Brown. 1969. Fungicide programs for the control of *Helminthosporium* leafspot and crown rot on Kentucky bluegrass. Plant Disease Reporter **53:** 462-466.

Couch, H.B. 1962. Diseases of turfgrasses. Reinhold Publishing Corporation, New York: United States of America. p. 62-63.

Couch, H.B. 1995. Diseases of turfgrasses, 3rd edition. Krieger Publishing, Florida: United States of America.

Craft, C.M. and E.B. Nelson, 1996. Microbial properties of composts that suppress damping-off and root rot of creeping bentgrass caused by *Pythium graminicola.* Applied and Environmental Microbiology **62:** 1550-1557.

Cubeta, M.A. and R. Vilgalys. 1997. Population biology of the *Rhizoctonia solani* complex. Phytopathology **87:** 480-484.

Davis, R.D. and B. Grof. 1994. Diseases of tropical pasture plants in southeast Asia and the Pacific. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 324-325.

Davis, R.D. and J.A.G. Irwin. 1994. Diseases of tropical pasture plants in Australia. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 259-262.

Davis, R.F., G.R. Noel and H.T. Wilkinson. 1994. Pathogenicity of *Tylenchorhynchus nudus* to creeping bentgrass. Plant Disease **78:** 169-172.

Dernoeden, P.H. 1993. Integrating strategies for the management of patch diseases caused by root invading ectotrophic fungi. In: B.B. Clarke and A.B. Gould (eds). Turfgrass patch diseases caused by ectotrophic root-infecting fungi. APS Press, Minnesota: United States of America. p. 124-130.

Dick, M.W., P.T.W. Wong and G. Clark. 1984. The identity of the oomycete causing 'kikuyu yellows', with the reclassification of the downy mildews. Botanical Journal of the Linnean Society **89:** 171-197.

Elliott, M.L. 1995. Effects of systemic fungicides on a Bernudagrass putting green infested with *Gaeumannomyces graminis* var. *graminis.* Plant Disease **79:** 945-949.

Emmons, R.D. 1995. Turfgrass science and management, 2nd edition. Delmar Publishers, New York: United States of America.

Fiddaman, P.J. and S. Rossall. 1995. Selection of bacterial antagonists for the biological control of *Rhizoctonia solani* on oilseed rape (*Brassica naptus*). Plant Pathology **44:** 695-703.

Fidanza, M.A. and P.H. Dernoeden. 1996. Brown patch severity in perennial ryegrass as influenced by irrigation, fungicide, and fertilizers. Crop Science **36:** 1631-1638.

Fraser, M.L., C.A. Rose-Fricker and W.A. Meyer. 1999. Registration of 'Matador' tall fescue. Crop Science **39:** 871-872.

Fraser, M.L., C.A. Rose-Fricker, W.A. Meyer and C.R. Funk.1999a. Registration of 'Wolfpack' tall fescue. Crop Science **39:** 872.

Fraser, M.L., C.A. Rose-Fricker, W.A. Meyer and C.R. Funk.1999b. Registration of 'Coronado Gold' tall fescue. Crop Science **39:** 1528.

Fravel, D.R. 1988. Role of antibiosis in the biocontrol of plant diseases. Annual Review of Phytopathology **26:** 75-91.

Fulkerson, B. and P. Wong. 1996. Kikuyu yellows (*Verrucalvus flavofaciens*) infection of kikuyu grass pastures. Research to farm. NSW Agriculture: Dairy Research and Development Corporation, Australia.

Giesler, L.J., G.Y. Yuen and G.L. Horst. 1996. The microclimate in tall fescue turf as affected by canopy density and its influence on brown patch disease. Plant Disease **80:** 389-393.

Hall, A.S. 1991. A study of pasture diseases in Natal. MSc. Thesis. University of Natal, Pietermaritzburg: South Africa.

Hall, A.S. 1992. Pasture grass diseases. In: T.N. Trench, D.J. Wilkinson and S.P. Esterhuysen (eds). South African plant disease control handbook: farmer support group. Kendall & Strachan, Pietermaritzburg: South Africa. p. 365-369.

Heimes, R. and F. Loecher. 1980. The possibility of controlling fairy ring and rust diseases in lawns using benodanil. In: J.B. Beard (ed). Proceedings of the third international turfgrass research conference. American Society of Agronomy, Madison: United States of America. p. 283-292.

Hill, W.J., J.R. Heckman, B.B. Clarke and J.A. Murphy. 1999. Take-all patch suppression in creeping bentgrass with manganese and copper. HortScience **34:** 891-892.

Hodges, C.F. 1994. Vegetative growth and sporulation of *Bipolaris sorokiniana* on sequentially older infected leaves of *Poa pratensis* exposed to postemergence herbicides. Mycopathologia **128:** 105-109.

Holmes, S.J.I. 1983. The susceptibility of agricultural grasses to pre-emergence damage by *Fusarium culmorum* and its control by fungicide seed treatment. Grass and Forage Science **39:** 209-214.

Howard, D.R. 1986. Brown patch. New Zealand Turf Management Journal **1:** 29-30.

Jackson, N. 1980. Yellow tuft disease of turfgrasses: a review of recent studies conducted in Rhode Island. In: J.B. Beard (ed). Proceedings of the third international turfgrass research conference, 11-13 July 1977, Munich: West Germany. American Society of Agronomy, Madison: United States of America. p. 265-269.

Jackson, N. 1993. Geographical distribution, host ranges, and symptomatology of patch diseases caused by soilborne ectotrophic fungi. In: B.B. Clarke and A.B. Gould (eds). Turfgrass patch diseases caused by ectotrophic root-infecting fungi. APS Press, Minnesota: United States of America. p. 17-40.

Kim, D.S., R.J. Cook and D.M. Weller. 1997. *Bacillus* sp. L324-92 for biological control of three root diseases of wheat grown with reduced tillage. Phytopathology **87:** 551-558.

Koch, E. 1999. Evaluation of commercial products for microbial control of soil-borne plant diseases. Crop Protection **18:** 119-125.

Kozelnicky, G.M. 1974. Updating 20 years of research "spring deadspot on bermudagrass". USGA Green Sec. Rec. **12:** 12-15. In: L.B. McCarty, L.T. Lucas and J.S. DiPaola (eds). 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortScience **27:** 1092-1093.

Krause, M., A. Nel and K. van Zyl. 1996. A guide to the use of pesticides and fungicides in the Republic of South Africa. National Department of Agriculture, Pretoria: South Africa. p. 126-142; 241-279.

Laing, M.D. 1997. Weeds, pests and diseases of turf. In: Groundsman's course July 1997. University of Natal, Pietermaritzburg: South Africa.

Lam, A. and G.C. Lewis. 1983. Chemical control of foliar diseases of perennial ryegrass (*Lolium perenne* L.) and their effects on yield and quality of the crop. Crop Protection **2:** 75-83.

Landschoot, P.J. and B.F. Hoyland. 1992. Grey leaf spot of perennial ryegrass turf in Pennsylvania. Plant Disease **76:** 1280-1282.

Lenné, J.M. 1994a. Diseases of *Centrosema*. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 43-60.

Lenné, J.M. 1994b. Diseases of other pasture grasses. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 169-194.

Lewis, G.C. 1985. Effect of soil-borne pathogens on ryegrass and white clover seedlings and their control. In: J.S. Brockman (ed). Weeds, pests and diseases of grassland and herbage legumes. British Crop Protection Council, Croydon: United Kingdom. p. 82-90.

Lo, C.T., E.B. Nelson and G.E. Harman. 1997. Improved biocontrol efficacy of *Trichoderma harzianum* 1295-22 for foliar phases of turf diseases by use of spray applications. Plant Disease **81:** 1132-1138.

Long, D.H., F.N Lee and D.O. TeBeest. 2000. Effects of nitrogen fertilization on disease progress of rice blast on susceptible and resistant cultivars. Plant Disease **84:** 403-409.

McCarty, L.B., L.T. Lucas and J.S. DiPaola. 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortScience **27:** 1092-1093.

McKirdy, S.J. and R.A.C. Jones, 1993. Occurrence of Barley Yellow Dwarf Virus serotypes MAV and RMV in over-summering grasses. Australian Journal of Agricultural Research **44:** 1195-1209.

McVickar, M.H. and J.S. McVickar. 1963. Approved practices in pasture management. The Interstate Printers & Publishers Danville, Illinois: United States of America. p. 270.

Mercer, P.C. and A. Ruddock. 1997. The effect of a single fungicide spray on yield and the control of disease of perennial ryegrass in an extended grazing regime. Annuals of Applied Biology **130:** 14-15.

Mohamed-Saleem, M.A. and K. Berhe, 1994. Diseases of tropical pasture plants in sub-saharan Africa. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 329-345.

Montealegre, J.R., M.A. Rojas., M.T. Varnero and E. Aballay. 1996. Effect of soil solarization on the control of *Sclerotium rolfsii* and nematodes in the Metropolitan Region of Chile. Fitopatologia **31**: 70-83

Moore, I. 1966. Grass and grasslands. Collins St James's Place, London: United Kingdom. p. 79-80.

Nel, A., M. Krause, N. Ramautar and K. van Zyl. 1999. A guide for the control of plant diseases. National Department of Agriculture, Pretoria: South Africa.

Peters, J.C. and M.W. Shaw. 1996. Effects of artificial exclusion and augmentation of fungal plant pathogens on a regenerating grassland. New Pytologistm **134:** 295-307.

Ranganathaiah, K.G. and Mathur, S.B. 1978. Seed health testing of *Eleusine coracana* with special reference to *Dreschlera nodulosa* and *Pyricularia grisea*. Seed Science Technology **6**: 943-951.

Roderick, H.W. and B.J. Thomas. 1997. Infection of ryegrass by three rust fungi (*Puccinia coronata, P. graminis* and *P. loliina*) and some effects of temperature on the establishment of the disease and sporulation. Plant Pathology **46:** 751-761.

Schroeder, C.B. and H.B. Sprague. 1996. Turf management handbook, 5th edition. Interstate Publishers, Illinois: United States of America. p. 5-147, 150-155.

Schultz, T.R., W.J. Johnston, C.T. Gotlob and J.D. Maguire. 1993. Control of ergot in Kentucky bluegrass seed production using fungicides. Plant Disease **77:** 685-687.

Shantz, H.L. and R.L. Piemeisel. 1917. Fungus fairy rings in eastern Colorado and their effect on vegetation. Journal of Agricultural Research **11:** 191-246.

Shurtleff, M.C. 1953. Susceptibility of lawn grasses to brown patch. Phytopathology **43:** 110.

Smiley, R.W., P.H. Dernoeden and B.B Clarke. 1992. Compendium of turfgrass diseases, 2nd edition. American Phytopathology Society, Minnesota: United States of America.

Smith, A.M. 1971. Control of spring deadspot of couch grass turf in New South Wales. J. Sports Turf Research Institute **47:** 60-65. In: L.B. McCarty, L.T. Lucas and J.S. DiPaola (eds). 1992. Spring dead spot occurrence in bermudagrass following fungicide and nutrient applications. HortScience **27:** 1092-1093.

Smith J.D., N. Jackson and A.R. Woolhouse.1989. Fungal diseases of amenity turf grasses. E. & F.N. Spong, London: United Kingdom. p. 401.

Spaull, A. 1985. Effects of nematodes on newly-sown ryegrass. In: J.S. Brockman (ed). Weeds, pests and diseases of grasslands and herbage legumes. British Crop Protection Council, Croydon: United Kingdom. p. 65-72.

Stahnke, G.K. 1993. First report of dollar spot, caused by *Sclerotinia homoeocarpa*, on turfgrass in Washington. Plant Disease **78:** 100.

Stanton, J.M. 1994. Nematode diseases. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 227-248.

Thompson, D.C., B.B. Clarke and J.R. Heckman. 1995. Nitrogen form and rate of nitrogen and chloride application for the control of summer patch disease in Kentucky bluegrass. Plant Disease **79:** 51-56.

Trevathan, L.E. 1982. Pathogenicity of ryegrass and cultural variability of Mississippi isolates of *Pyricularia grisea*. Plant Disease **66:** 592-594.

Trevathan, L.E., M.A. Moss and D. Blasingame. 1994. Ryegrass blight. Plant Disease **78**: 113-117.

Trutmann, P. 1994a. Diseases of tropical pasture plants in central and South America. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 302-306.

Trutmann, P. 1994b. Management of diseases of tropical pasture plants. In: J.M. Lenné and P. Trutmann (eds). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 349-363.

Turgeon, A.J. 1991. Turfgrass management, 3rd edition. Regents/Prentice Hall, New Jersey: United States of America.

Uddin, W., M.D. Soika, F.E. Moorman and G. Viji. 1999. A serious outbreak of blast disease (gray leaf spot) of perennial ryegrass in golf course fairways in Pennsylvania. Plant Disease **83**: 783.

Uddin, W., G. Viji, G.L. Schumann and S.H. Boyd. 2003. Detection of *Pyricularia grisea* causing grey leaf spot of perennial ryegrass turf by a rapid immuno-regregnition assay. Plant Disease **87:** 772-778.

Van Bezooijen, J. 1985. Pest and disease problems in newly sown grass in the Netherlands. In: J.S. Brockman (ed). Weeds, pests and diseases of grassland and herbage legumes. British Crop Protection Council, Croydon: United Kingdom. p. 57-64

Vengris, J. and W.A. Torello. 1982. Lawns: basic factors, construction and maintenance of fine turf areas, 3rd edition. Thomson Publications, California: United States of America.

Welty, R.E. and R.E. Barker. 1993. Reaction of twenty cultivars of Tall fescue to stem rust in controlled and field environments. Crop Science **33:** 963-967.

Wong, P.T.W. 1975. Kikuyu yellows - a disease cause by an undescribed Phycomycete. Plant Disease Reporter **59:** 800-801.

Wong P.T.W. and L.A. Tesoriero, 1990. Evaluation of fungicides for the control of kikuyu yellows (*Verrucalvus flavofaciens*). Plant Protection Quarterly **5:** 76-77.

Yaegashi, H. and S. Udagawa. 1978. The taxonomic identity of the perfect state of *Pyricularia grisea* and its allies. Canadian Journal of Botany **56:** 180-183.

York, K. 1996. Take-all patch in cereals and amenity turf. International Turfgrass Bulletin **193:** 29-30.

Yuen, G.Y., M.L. Craig and L.J. Giesler. 1994. Biological control of *Rhizoctonia solani* on tall fescue using fungal antagonists. Plant Disease **78:** 118-122.

Zeiders, K.E. 1984. *Helminthosporium* spot blotch of switchgrass in Pennsylvania. Plant Disease **68:** 120-122.

Zhang, Z. and G.Y. Yuen. 1999. Biological control of *Bipolaris sorokiniana* on tall fescue by *Stenotrophomonas maltophilia* strain C3. Phytopathology **89:** 817-822.

CHAPTER 3

TURFGRASS AND PASTURE PRODUCTION SURVEY

ABSTRACT

A survey was conducted in 1999/2000 to determine management and maintenance practices implemented by pasture farmers and groundsmen in KwaZulu-Natal. Farmers and groundsmen where questioned on soil and topdressings, pasture and turfgrass cultivars, irrigation and drainage, fertilization and liming, maintenance and weed, pest and disease control. These responses are recorded as pie charts. Emphasis was placed on determining insect and disease occurrences and control measures implemented, highlighting the possible implementation of biological control. Linear Regression and Chi-square tests were also used to identify significance ($P \leq 0.05$). Significance was noted only for the pasture survey in terms of fertilization and the incidence of diseases and weeds; and for grazing and decreased weed, insect and kikuyu yellows incidences. Survey responses in terms of disease and insect incidences, indicated that to the farmer this is of little concern and chemical control measures are not warranted, based on expense and potential residual effects. On the other hand, disease and insect occurrences are of a major concern to groundsmen. Caterpillars, crickets and termites were identified as the most frequently encountered insects and the fungal disease, dollar spot is the most severe disease. In terms of implementing biological control, responses indicated that the majority of managers would use biological control if more was known about the methods of application and levels of success that can be achieved. At present, there is much intellectual knowledge about biological control, with only very small amounts of this knowledge filtering to those who will apply the science. The future success of biological control is therefore dependent on the relationship between researchers (academics) and farmers or groundsmen.

3.1 INTRODUCTION

Improvement of management systems is essential for both economic and environmental stability. Management is, however, variable depending on environmental conditions and the knowledge and experience of the farmer or groundsman. Management practices impact largely on pest incidences and severity, with new diseases frequently being encountered. Control methods are variable. These methods are aimed at increasing the quantity and quality of plant products, thus increasing their availability and use. Due to growing environmental concern and an increase in pesticide resistance, there has been renewed interest in the use of natural enemies (biological control) for disease control.

A survey was compiled and mailed to dairy/cattle farmers surrounding Pietermaritzburg and to groundsmen in the KwaZulu-Natal region to determine their management principles and to ascertain pest problems and control measures implemented. The survey also addressed the perception that farmers and groundsmen have of biological control, and if they would implement such a control system.

3.2 MATERIALS AND METHODS

The survey questionnaire for both farmers and groundsmen was compiled based on a literature survey (Chapters 1 and 2) and input from extension officers of the Pastures Section, Department of Agriculture and Environmental Affairs, Cedara KwaZulu-Natal[1].

[1] KwaZulu-Natal Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200, South Africa. Tel: (+27) 33 3559100

3.2.1 PASTURE PRODUCTION SURVEY

A pasture survey (Appendix 2) addressing management and pest incidences, and the farmer's perception of biocontrol, was mailed to farmers registered with the Milk Producer's Organisation of Pietermaritzburg[2]. An addressed reply envelope was included in the mailed survey.

3.2.2 TURF PRODUCTION SURVEY

A turf survey (Appendix 3) addressing management and pest incidences, and the groundsmen's perception of biocontrol, was mailed to groundsmen registered with the Turfgrass Manager's Association of KwaZulu-Natal[3] extending from the South Coast of KwaZulu-Natal to the Drakensberg. An addressed reply envelope was included in the mailed survey.

3.2.3 STATISTICAL ANALYSIS

Significant ($P=\leq 0.05$) relations in the data were determined using simple Linear regressions and Chi-square tests, in Genstat 5 (Anon, 2000).

[2] Milk Producers Association, P.O. Box 604, Pietermaritzburg, 3200, South Africa. Tel: (+27) 33 345 4525.

[3] Turfgrass Managers Association, P.O. Box 35620, Northway, 4065, South Africa.

3.3.1 RESULTS OF THE PASTURE PRODUCTION SURVEY

A total of 57 replies were received. However, not all questions were answered in the surveys. Responses were thus analyzed for the response obtained for each question. Survey responses are visually represented as pie charts. Pie charts allow for the representation of a single data series, where each value is represented as a slice.

MANAGEMENT

3.3.1.1 Soil forms

Soil types encountered by the farmers are summarized in Figure 3.1.The majority of soil types recorded in the survey were deep red and heavy clay soils (each totaling approximately 30% of responses). Humic/loamy soils were encountered by approximately 19% of the farmers, black soils approximately 7%, thin on shale by 11% and sandy soils by approximately 4% of the farmers.

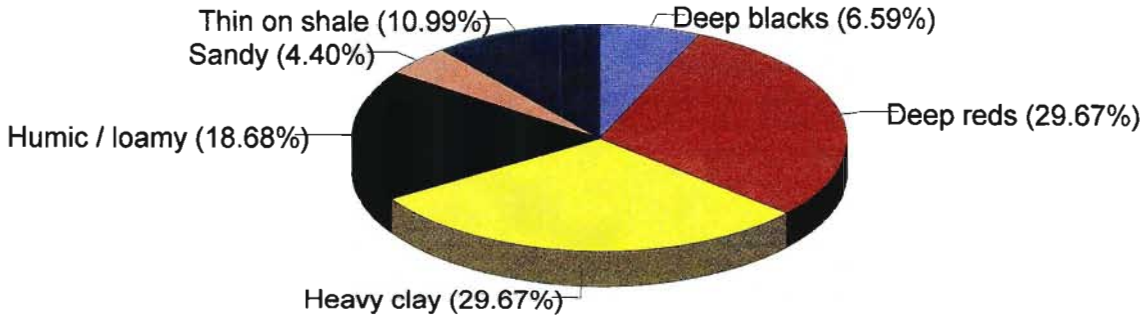


Figure 3.1 Commonly occurring soil types identified by the KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

3.3.1.2 Pasture grasses species

Pasture grass species included in the survey and the frequency of their use, expressed as a percentage of the overall pasture responses, are shown in Figure 3.2 (Refer to Appendix 4 for a list of common and botanical names of grasses referred to in the survey). Commonly cultivated pasture grasses in the KwaZulu-Natal Midlands were, kikuyu (25%); annual ryegrass (24%); perennial ryegrass (10%); weeping love grass (7%) and tall fescue (6%). Leguminous plants have the potential to improve pasture potentials. Clover was the more commonly utilized legume species, with a survey response of 10% utilization, i.e., 7% white and 3% red clover.

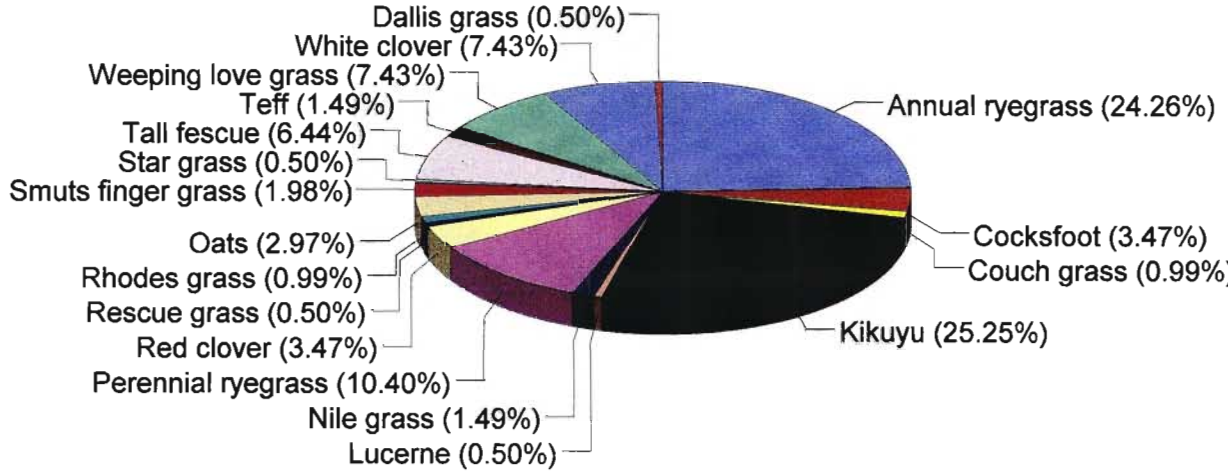


Figure 3.2 **Pasture species utilized in the establishment of pastures by KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).**

3.3.1.3 Irrigation

Of the farmers who responded to the questionnaire, only 2% did not supplement with irrigation on their pastures. Figure 3.3 summarizes the irrigation systems used by the remaining 98%. Of this 98%, 95% indicated that they use a non-mechanized sprinkler, i.e. overhead sprinkler and dragline systems. Only 2% of the farmers indicated that they use a mechanized sprinkler system, i.e., center pivot.

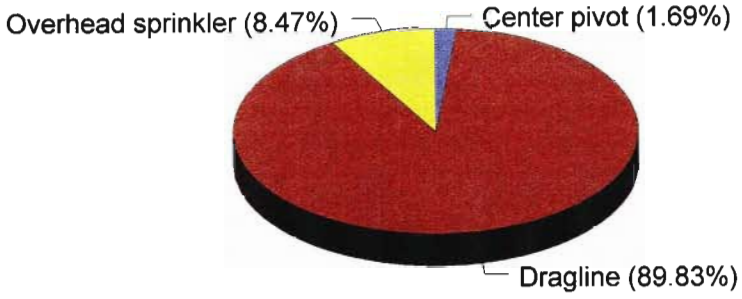


Figure 3.3 Irrigation systems utilized by the KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

Of the farmers, 77% indicated that they had consulted an irrigation specialists. Table 3.1 summarises the farmers responses to water use efficiency.

Table 3.1. Summary of KwaZulu-Natal Midlands farmer's responses to important factors needing consideration when implementing an irrigation program on pastures. From a pasture production survey (1999/2000)

Factors of consideration	Farmer's response (%[a])	
	Taken into consideration	Not considered
Root depth of the pasture	58	35
Water-holding capacity of the soil	63	40
Topography, i.e. land slope	19	65
Expected evaporation losses	58	49
Effectiveness of your irrigation system	88	26

[a] Not all farmers responded to each question posed, therefore the response has been taken as a percentage of the average number of responses (43 farmers) recorded for this table.

3.3.1.4 Fertilization and liming

Of the surveyed farmers, 89% indicated that fertilization and liming practices were based on prior soil analysis (approximately 6 weeks before planting).

Figure 3.4 indicates that at least 22% of the total fertilizer application by the surveyed farmers was dedicated to liming, with 16% of the total amount of fertilizers and lime applied, attributed to dolomitic lime. Approximately 9% of the farmers indicated that they did not apply lime.

In terms of a Phosphorus (P) source, 53% farmers used diammonium phosphate. This accounts for approximately 13% of total fertilizers used by farmers; monoammonium phosphate accounted for 7% and superphosphate for 5% of total fertilizers used. Of the surveyed farmers, 88% used potassium chloride (accounted for 18% of the total fertilisers used). Approximately 52% of the surveyed farmers used urea (accounts for 15% of the total fertilizers used) as an N source, with limestone ammonium nitrate (LAN) (accounting for 12% of the total fertilizers used) applied by 42% of the farmers.

Of the surveyed farmers, 91% indicated that they applied split dressings of N, over an annual application. Approximately 5% of pasture fertilization was dedicated to fertilizer mixes, with 2% of the total 5% of mixes used, containing zinc (Zn). Three of the total surveyed farmers (accounting for 1%) used chicken litter or animal manure. These three responses were associated with the use of the fertilizer mixes.

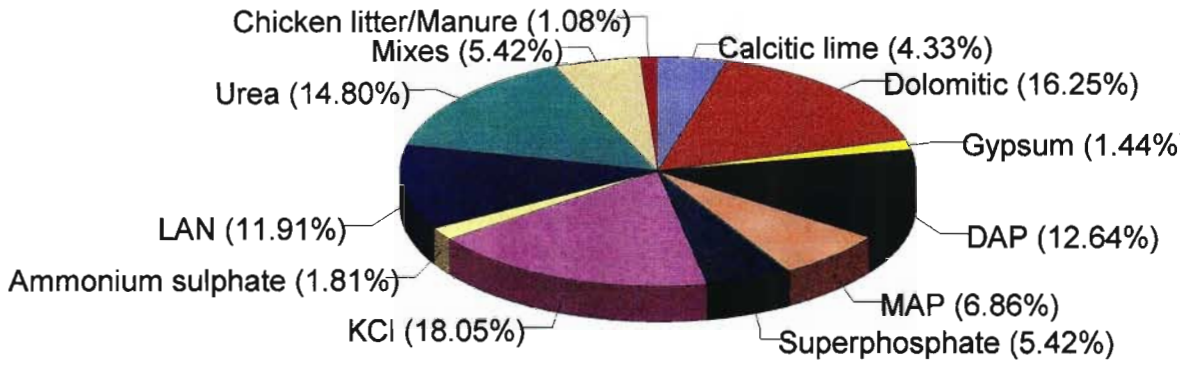


Figure 3.4 Fertilizers and lime utilized by KwaZulu-Natal Midlands farmers for luxurious pasture growth. From a pasture production survey (1999/2000).

3.3.1.5 Grazing programs

Figure 3.5 indicates the preference of KwaZulu-Natal Midlands farmers for rotational grazing. The most popular method of rotational grazing was strip grazing (accounting for over 55% of the recorded responses), followed by rotational blocks. A 5% response was received for the rotational wagonwheel grazing system.

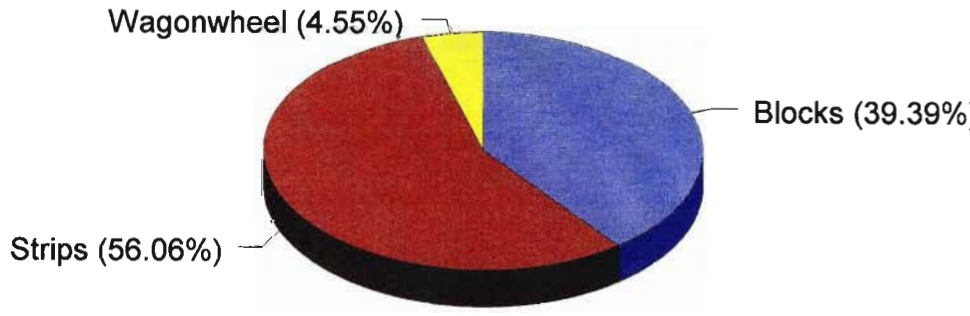


Figure 3.5 Rotational grazing systems commonly implemented by KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

Table 3.2 summarizes the grazing management of pastures.

Table 3.2. Summary of KwaZulu-Natal Midlands farmer's responses to questions asked in terms of grazing management. From a pasture production survey (1999/2000)

Questions	Farmer's response (%[a])	
	Yes	No
Do you graze different classes of animals on a pasture?	49	51
Do you allow perennial pastures to grow out to the flowering stage?	33	67
Do you use a loaf camp in your rotational program?	30	70
Do you apply clean-up cuts with a mower:		
i. to remove uneaten stem material after grazing?	94	6
ii. for haymaking when growth is in excess?	70	30

[a] Not all farmers responded to each question posed, therefore the response was determined as a percentage of the average number of responses recorded for each question

3.3.1.6 Weed management

Weeds commonly encountered by KwaZulu-Natal Midlands farmers are shown in Figure 3.6. Grass species accounted for almost 50% of the weeds encountered, i.e. 19% annual and 30% perennial weeds. Broadleaf weeds, attributed 28% (including thistle dock), and nutgrass/nutsedge attributed 22% and was identified as severe in terms of occurrence.

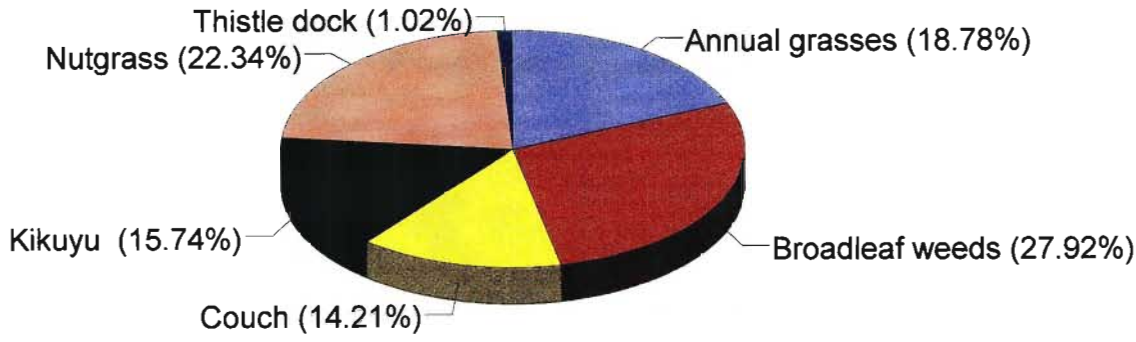


Figure 3.6 Weeds commonly encountered in the pastures of KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

Figure 3.7 outlines cultural methods of weed control as identified in the survey. Of the surveyed farmers, 23% indicated that they used physical labour, 40% indicated that they implemented a carefully managed grazing system and 36% indicated that they mow. On the whole, cultural methods of control were most commonly implemented by the farmers with a 98% response from the surveyed farmers, in comparison to 88% response received for herbicide usage.

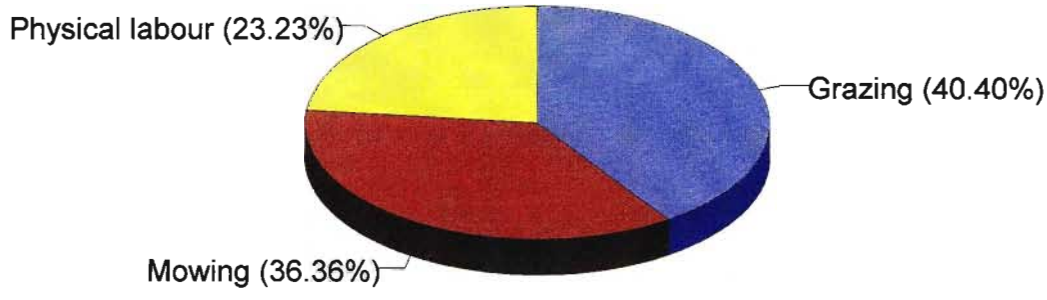


Figure 3.7 Cultural methods of weed control implemented by KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

Figure 3.8 summarises herbicide usage of surveyed farmers in the KwaZulu-Natal Midlands (Refer to Appendix 5 for a list of trade names and active ingredients of herbicides used). Approximately 61% of the farmers used 2,4-D for weed control (including 2,4-D/Dicamba/MCPA), 24% of the farmers indicated that they use MCPA based herbicides. Only 1 response was received for Benzioxide and EPTC. Glyphosate (round-up) (more frequently used with a 4% survey response) and Imazapyr offer pre-emergent weed control. The survey showed a 7% response for the control of shrubs/trees using Triclopyr.

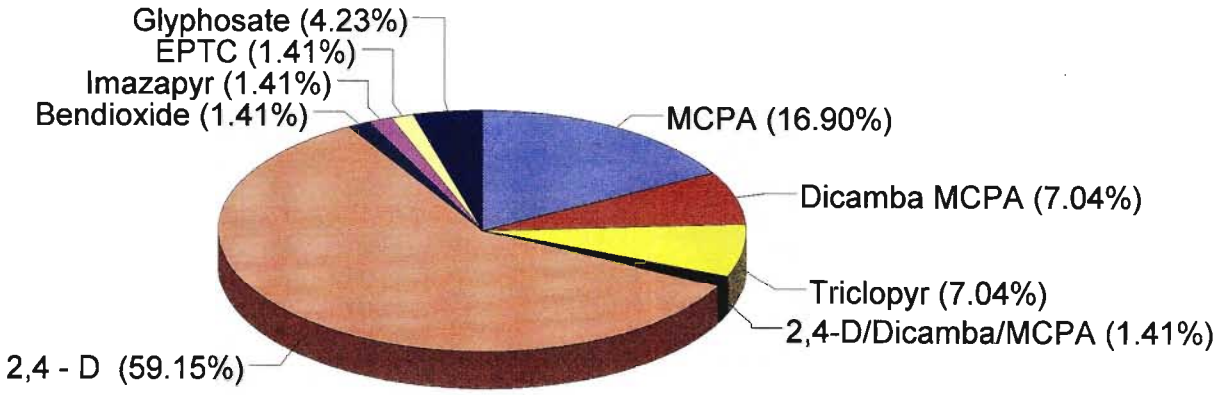


Figure 3.8 Herbicide use as identified by KwaZulu-Natal Midlands farmers for the control of weeds. From a pasture production survey (1999/2000).

INSECT AND DISEASE CONTROL

3.3.1.7 Insects and other pest management

Figure 3.9 summarises the insect pests encountered by KwaZulu-Natal Midlands farmers. Many of the farmers (39%) indicated that insect pests were not evident or severe enough to be given much consideration in terms of implementing control measures.

Insect pests recorded as being most severe were: aphids (6%); birds (6%); caterpillars (7%); ground weevils (4%); snails and slugs (5%) and rats/mice (5%). Other commonly encountered insect pests (greater than 5% occasional occurrence), were armyworms (9%) and bollworms (6%). Armyworms, although not considered severe, were identified as critical to control.

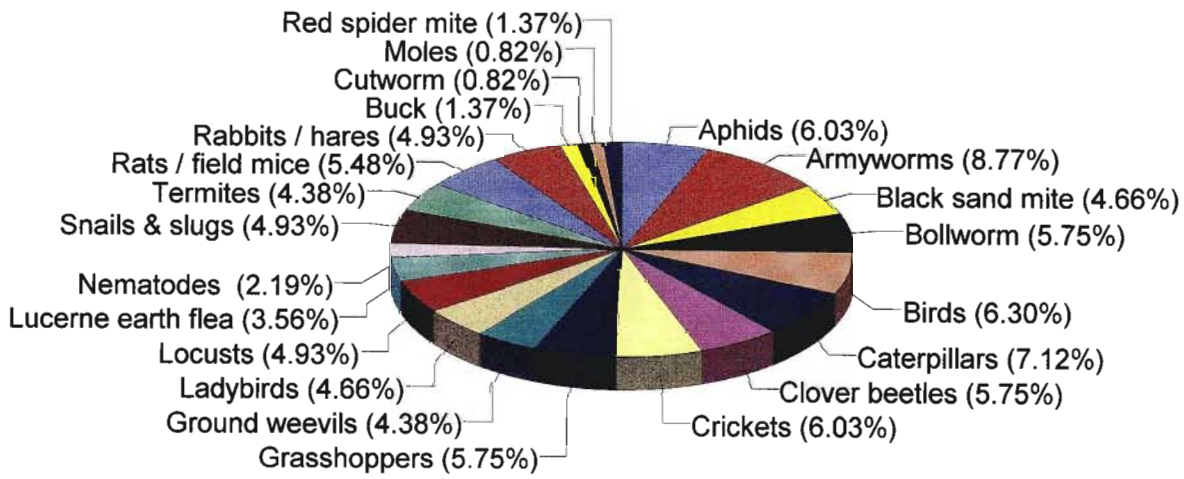


Figure 3.9 Insects and other pests commonly encountered by KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

Of the farmers only 17 (30%) responded as implementing any form of control against insect pests in their pastures, reasons being much the same as for the limited use of herbicides as mentioned before. Only two farmers (4% of the total farmers surveyed) indicated that they used cultural practices. Cultural control of insect pests included grazing and trampling, cutting for hay/silage and timing of land preparation. In terms of biological control there were also only two responses. One farmer indicated the use of natural/biological control by means of encouraging bird activities rather than identifying the birds as pests.

Of the surveyed farmers, only 14 (25%) indicated that they used chemicals as a means of pest control. Figure 3.10 shows the pesticide active ingredients used (Refer to Appendix 6 for a list of trade names and active ingredients of insecticides used). Lambda-cyhalothrin (trade name, karate) was identified as the most commonly used pesticide, accounting for 25% of pesticides used. Other commonly used insecticides included, permethrin (19%), cypermethrin (13%) and *Bacillus thurienginsis* EG2215 (13%).

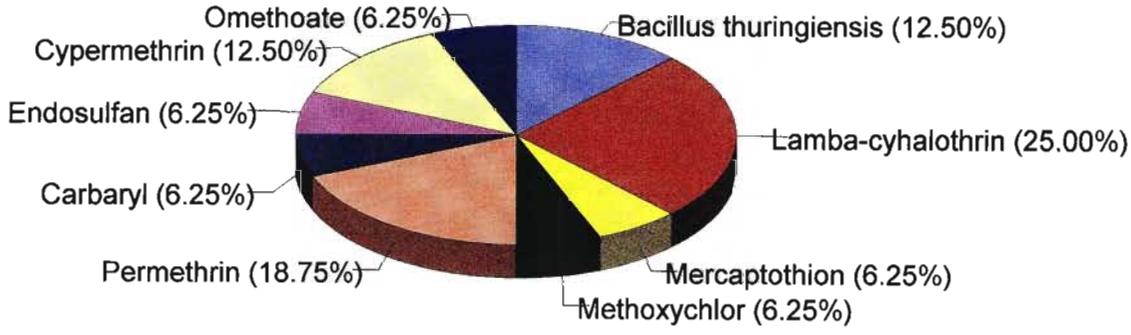


Figure 3.10 Insecticide use as identified by KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

3.3.1.8 Disease management

Figure 3.11 summarises diseases encountered by KwaZulu-Natal Midlands farmers included in the survey. Overall diseases were not prevalent or of no major concern.

Severe outbreaks of kikuyu yellows, rust (together accounting for 60% of diseases encountered) and seedling death (11%) were noted by farmers. Diseases having a direct pathological effect on the animals grazing the infected pasture are ryegrass toxicity and ergot (each accounting for 5% of the total diseases encountered), as well as choke (3%). Ryegrass blast, which accounted for 4% of the total diseases, is a fairly new disease.

Approximately 79% of the surveyed farmers indicated that they diagnosed disease occurrence themselves.

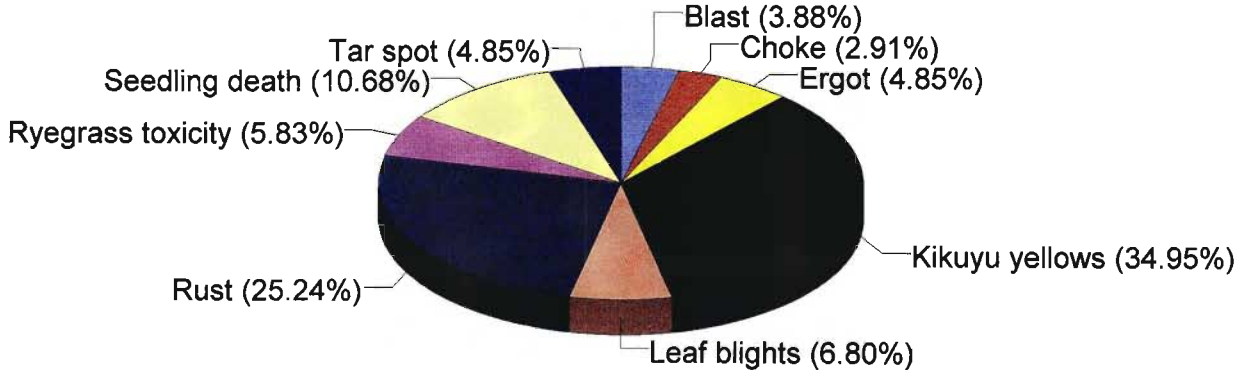


Figure 3.11 Pasture diseases commonly encountered by KwaZulu-Natal Midlands farmers. From a pasture production survey (1999/2000).

The choice of control measures presented to the farmers and their responses are summarized in Table 3.3.

Table 3.3 Summary of KwaZulu-Natal Midlands farmer's response to disease control methods implemented. From a pasture production survey (1999/2000)

Disease control methods	Farmer's response	
	Yes (%)	Response
CHEMICAL CONTROL	11	Chemicals identified (Trade name & Active ingredient): • Bravo (chlorothalonil) • Punch (carbendaziml/flusilazole) • Ridomil (metalaxyl) • Tilt (propiconazole)
CULTURAL PRACTICES	9	Methods identified: • grazing • irrigation • mowing • quarantine • replanting
BIOLOGICAL CONTROL	0	
INTEGRATED CONTROL	0	

3.3.1.9 Understanding of biological control and proposed use

In terms of biocontrol potential, 61% of the surveyed farmers indicated that they would consider implementing biocontrol. Of these, 51% indicated that they had no existing knowledge of biological control.

3.3.2 RESULTS OF THE TURF PRODUCTION SURVEY

A total of 13 replies were received, 11 of which were from golf course groundsmen, and one each from a race track and sportsfield manager.

MANAGEMENT

3.3.2.1 Soils and Topdressing

The predominant soil type identified in the survey was sand.

Figure 3.12 summarises the topdressings used by the surveyed groundsmen. The most commonly used topdressings were sterilized sand (32%), pine bark (24%) and animal manure (12%).

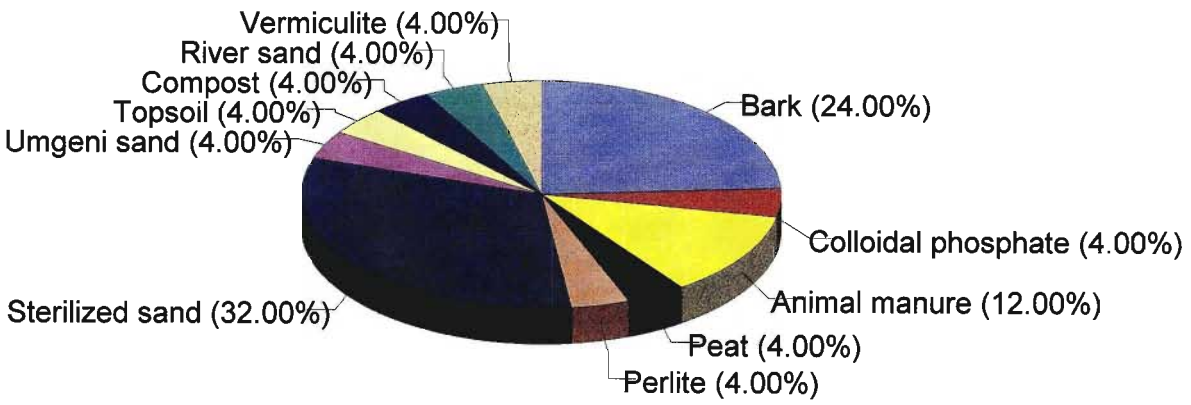


Figure 3.12 Topdressings used by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Approximately 62% of the groundsmen topdressed lightly at 1-10mm. The frequency/timing of topdressing is summarized in Table 3.4.

Table 3.4 Summary of KwaZulu-Natal groundsmen's responses as to frequency of topdressing. From a turf production survey (1999/2000)

When to topdress? Factors to consider	Response recorded (%)[b]
Irrigation	8
Thatch	8
Frequency	
Two or three times a month	15
Once a month	8
Twice per annum	23
Three times per annum	15
Four times per annum	8
Annually	23

[b] Expressed as a percentage of the total responses received from the survey. Note that some groundsmen offered more than one option to the frequency of topdressing.

3.3.2.2 Greens base structure

Of the surveyed golf courses, 73% of the groundsmen indicated that they used local specifications as the base for greens.

3.3.2.3 Turf grasses species

Figure 3.13 summarises grass species used for green establishment (Refer to Appendix 4 for a list of common and botanical names of grasses referred to in the survey). Country Club and Bermuda grass (each accounting for 38% of the 11 golf course responses) were the more commonly used green grasses. Bayview and Lm grass(each accounting for 7% of the 11 golf course responses) are species commonly planted as a mixture with *Cynodon dactylon.*

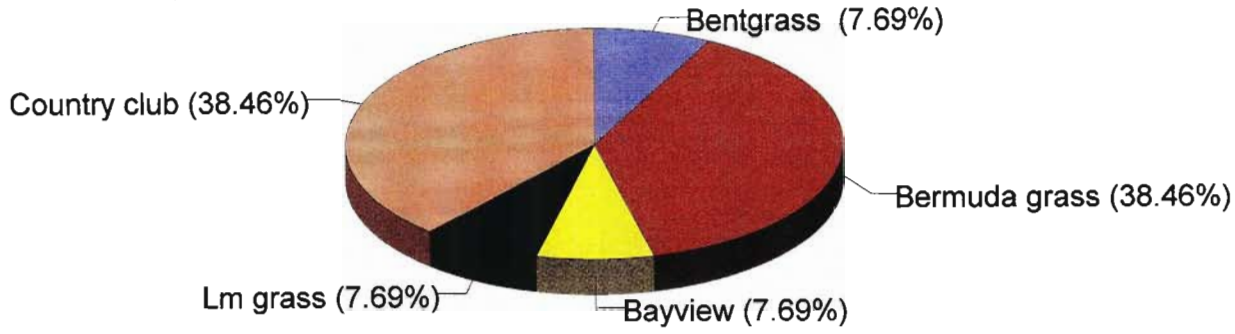


Figure 3.13 Turfgrass cultivars used in golf green establishment by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

In the establishment of golf tees and fairways, Kikuyu (*Pennisetum clandestinum*) and *Cynodon* sp. were the grass species listed, with mention given to Buffalo grass (*Stenophrum secundatum*).

Kikuyu is also used in the establishment of race courses as indicated by the single response received.

3.3.2.4 Irrigation

The survey indicated that golf greens, accounted for 73% of fixed irrigation allocations.

Figure 3.14, shows irrigation scheduling used by the surveyed groundsmen. General observation tools used to monitor irrigation requirements were a tensiometer (17%), simple visual observations and weather monitoring (33%), electronic scheduling (33%) and an evaporation pan (17%).

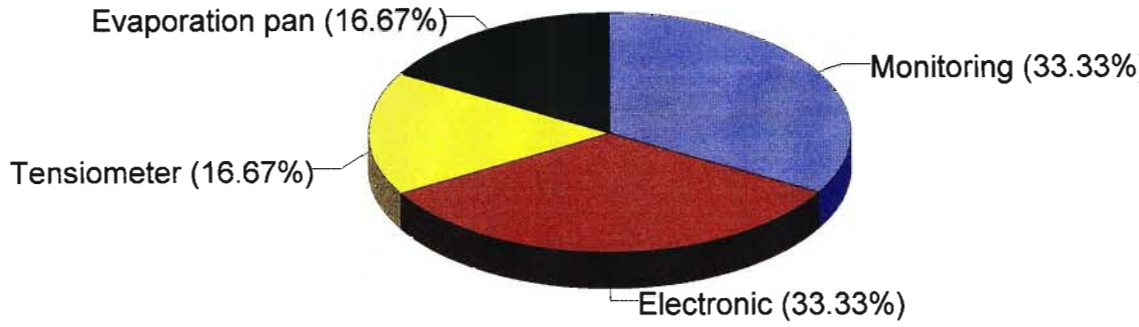


Figure 3.14 Irrigation scheduling as identified by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Figure 3.15 summarises the groundsmen's responses to the irrigation equipment used. Draglines and pop-ups (together attributing 86% to irrigation) were the more frequently used systems. Boomsprayers (7%) were identified for fairway use.

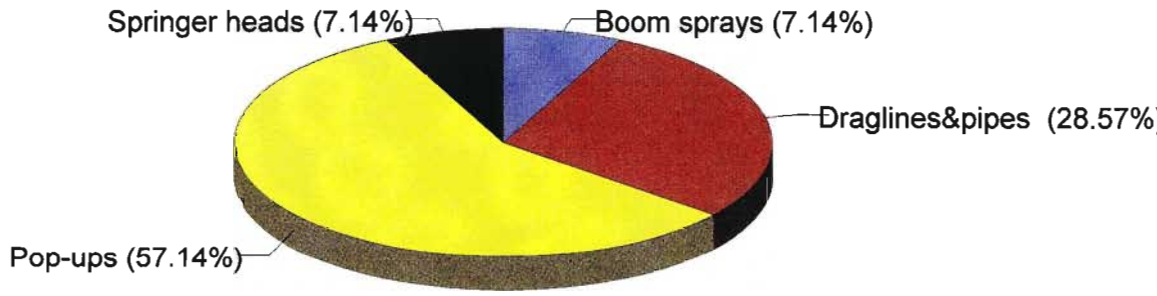


Figure 3.15 Irrigation systems/equipment used by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Figure 3.16 summarises the time as to when groundsmen irrigate. Early morning irrigation received the highest response. While, 30% of the surveyed groundsmen irrigated at midday, 26% irrigated in the late afternoons and only 4% irrigated at night.

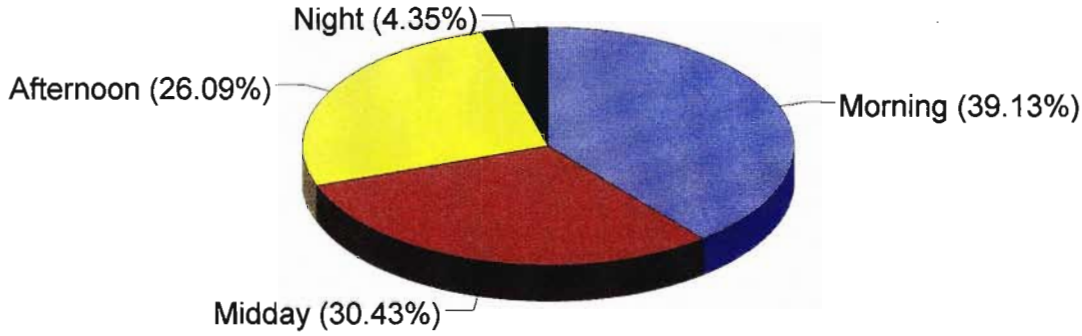


Figure 3.16 Timing of when to irrigate as implemented by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Figure 3.17 represents the drainage systems used by the surveyed KwaZulu-Natal groundsmen. A sand or drainage system (of which sand is a vital component) comprised 64% of the responses received. A herringbone system was also popular, with a 21% response.

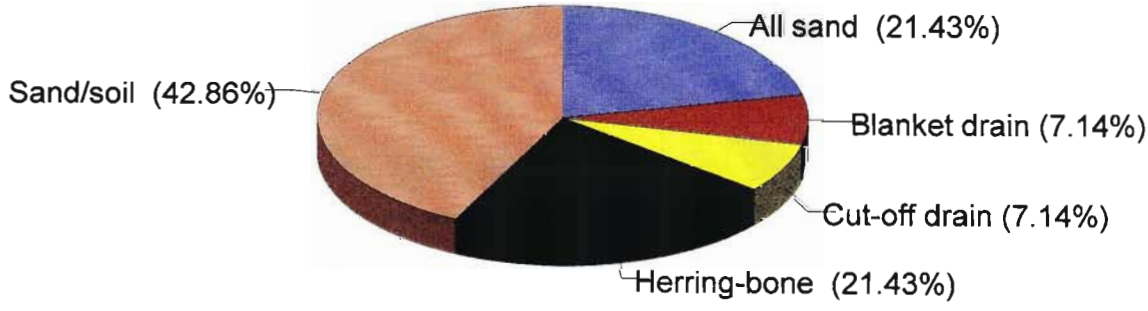


Figure 3.17 Drainage systems commonly used by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

3.3.2.5 Fertilization

Fertilizers are applied in either a granular or a liquid form, with a greater survey response received for the granular form.

Figure 3.18 summarises the fertilization methods used by the surveyed KwaZulu-Natal groundsmen. Granular fertilizers and lime are best applied with a spreader, such as a Walk behind spreader (6%), a Granular spreader (6%), Hopper (6%), Spandicar (18%) or by hand (12%). Liquid fertilizers (fertigation) received a 46% response.

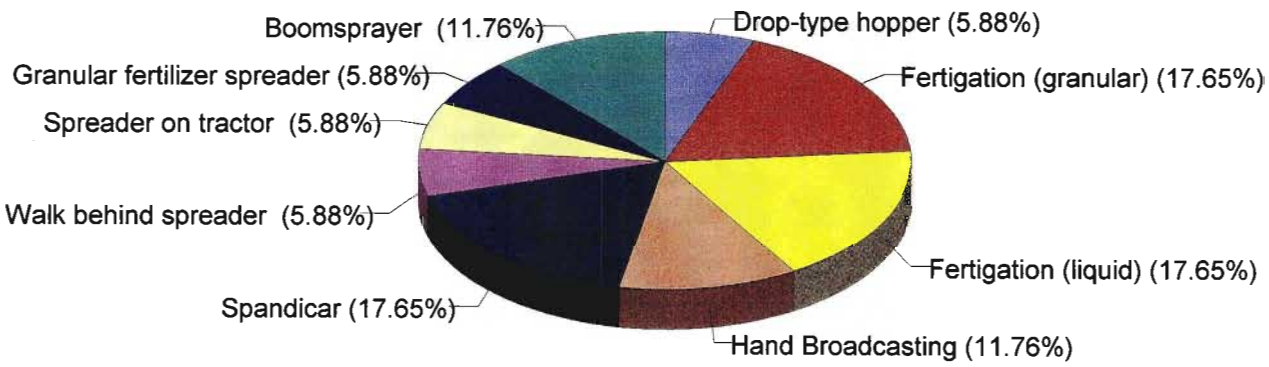


Figure 3.18 Methods of fertilizer application implemented by KwaZulu-Natal groundsmen. From a turf production survey.

3.3.2.6 Mowing

Figure 3.19 summarises mowers used by the surveyed KwaZulu-Natal groundsmen. Jacobson Greens King IV (11%) and the Toro 3200 (19%) were the more commonly used mowers. Toro GM 1000 (11% response) was commonly used on greens. On golf fairways, a 5 random gang tow behind attached to a tractor (11%) and the Toro 6500D mower (8%) were used. The 5 random gang tow behind (Ransome) and a Cylinder mower (3%) were also identified for use on race tracks.

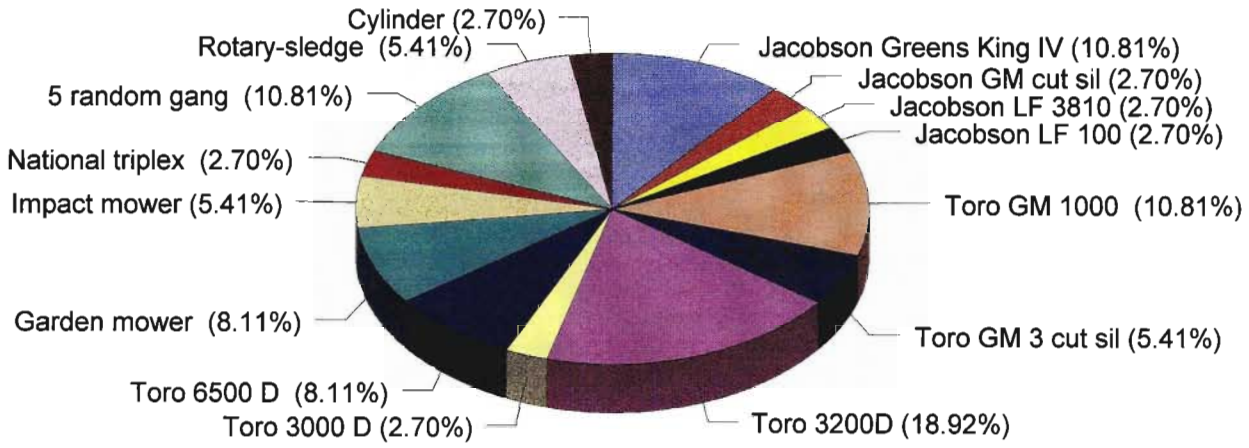


Figure 3.19 Commercially available mower types used by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Table 3.5 summarises the groundsmen's responses to the frequency of mowing.

Table 3.5 Summary of responses given by KwaZulu-Natal golf course groundsmen as to their mowing frequency. From a turf production survey (1999/2000)

MOWING FREQUENCIES	RECORDED RESPONSES (%)[c]		
	greens	tees	fairways
7 times a week	55	9	9
6 times a week	27	0	0
3 times a week	18	18	18
2 times a week	18	36	36
1 time a week	0	36	36
Every 2 weeks	0	0	9
Only when necessary	9	0	0
Never (refers specifically to winter)	0	0	9

[c] A total of 11 responses were received from golf course managers and the percentages above have been calculated as a percentage of this total.

Figure 3.20 summarises the mowing heights for golf courses. On average a height of 3-4mm was ideal for golf greens, 6-9mm on tees and 14-16mm on fairways.

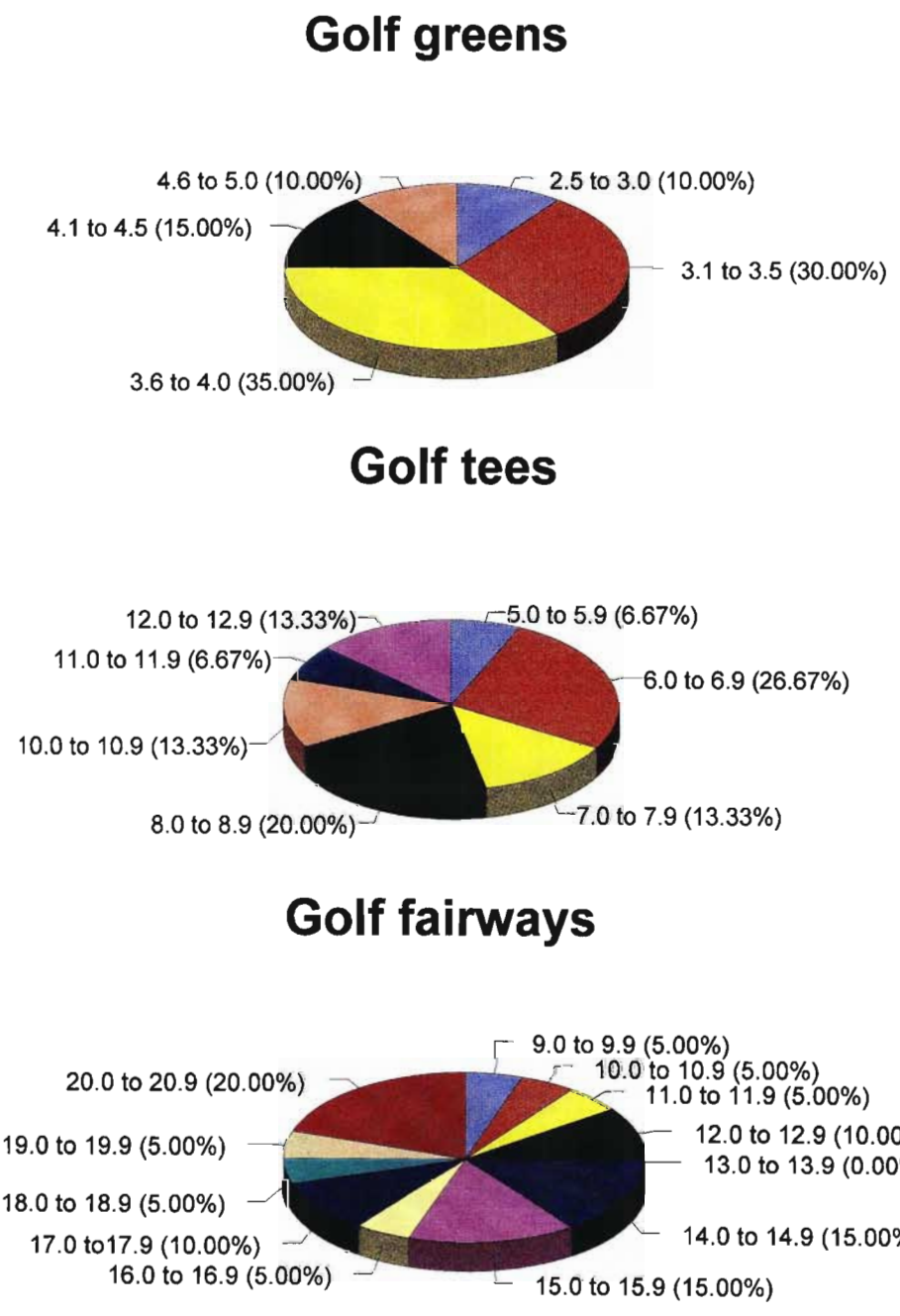


Figure 3.20 Mowing heights (mm) of golf greens, tees and fairways implemented by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

3.3.2.7 Decompaction and Aeration

Figure 3.21 summarises methods implemented by surveyed KwaZulu-Natal groundsmen to reduce thatch. Hollow tyning was the more frequently used method, accounting for almost half of the responses received (46%). Solid tyne spiking (19%) was also implemented. Coring (15%) is essentially hollow tyning. Verti-draining (15%) is the quickest and easiest means of relieving decompaction.

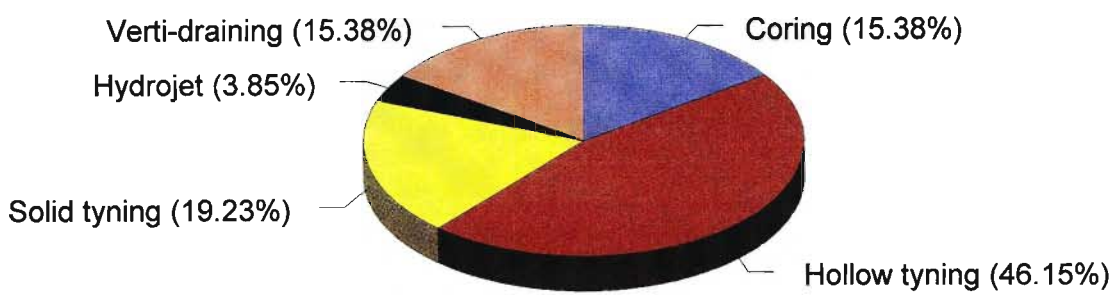


Figure 3.21 Methods by KwaZulu-Natal groundsmen to decrease compaction and increase aeration. From a turf production survey (1999/2000).

3.3.2.8 Weed management

Figure 3.22 represents the weeds commonly encountered by the surveyed groundsmen. Commonly encountered weeds were the various clover species (19%), grass invaders (15%) and watergrass (*Cyperus* spp.) (13%). Some groundsmen identified weeds as a serious problem, while 8% indicated that they had no weed problems and 23% indicated only minor weed occurrences.

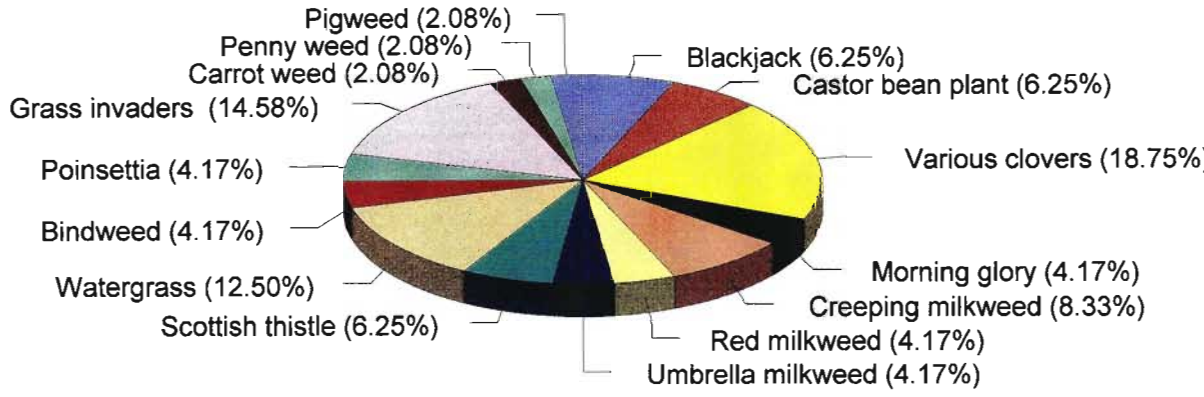


Figure 3.22 Weeds commonly encountered by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Twelve of the 13 surveyed KwaZulu-Natal groundsmen indicated herbicide use to varying degrees. Figure 3.23 summarises the specific herbicides used (Refer to Appendix 5 for a list of trade names and active ingredients of herbicides used). Methyl bromide, was the only soil fumigant identified, accounting for 2% of herbicide use. Broad spectrum control was attributed to Glyphosate (23%) and MSMA (16%). Watergrass control was achieved with EPTC and Halosulfuron (2% each). Selective herbicides identified included 2,4-D/Dicamba/MCPA (14%), Bendioxide (9%), 2,4-D and MCPA (7%), Dicamba (8%) and Atrazine, Cycloxydim and Propyzamide (2% each).

Approximately 85% of the KwaZulu-Natal groundsmen used cultural control, being either mowing and/or labour for hand weeding.

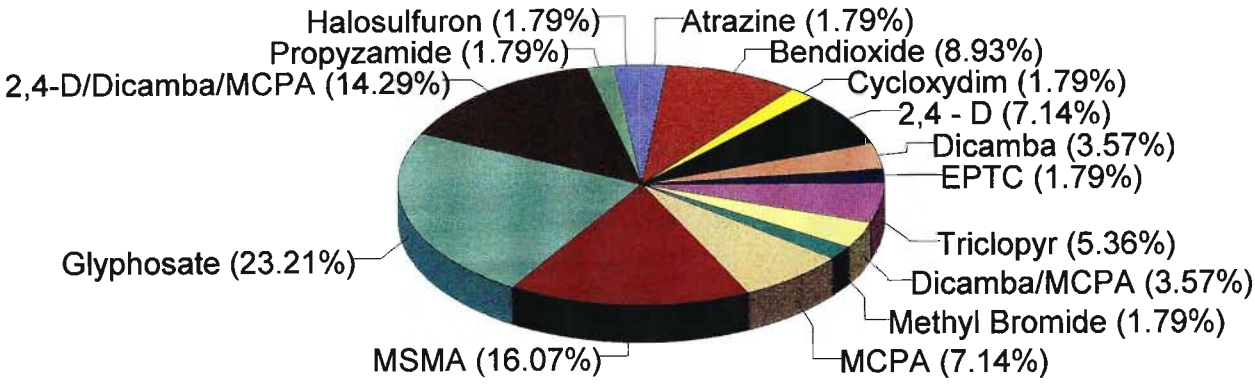


Figure 3.23 Herbicides used by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

INSECT AND DISEASE CONTROL

3.3.2.9 Insects and other pest management

Figure 3.24 represents the pests commonly encountered by the surveyed groundsmen.

Garden moles have been included under pests based on the kingdom classification, system 1 where organisms are classified either under animals or plants (Keeton, 1976).

In turf, caterpillars (including armyworms, burrowing caterpillars, lawn caterpillars, cutworms, grass bag worms and sod web worms) accounted for 32% of pests encountered in the survey area. Crickets and termites also accounted for a large percentage, being that of 17% and 15%, respectively. Black maize beetles (8%) were identified as insects feeding on stems and organic matter, with white grubs (6%) feeding only on organic matter. Nematodes (9%) predominately cause damage to grass roots. Sap-sucking insects (9%) identified included mites, leafhoppers, aphids and scale insects.

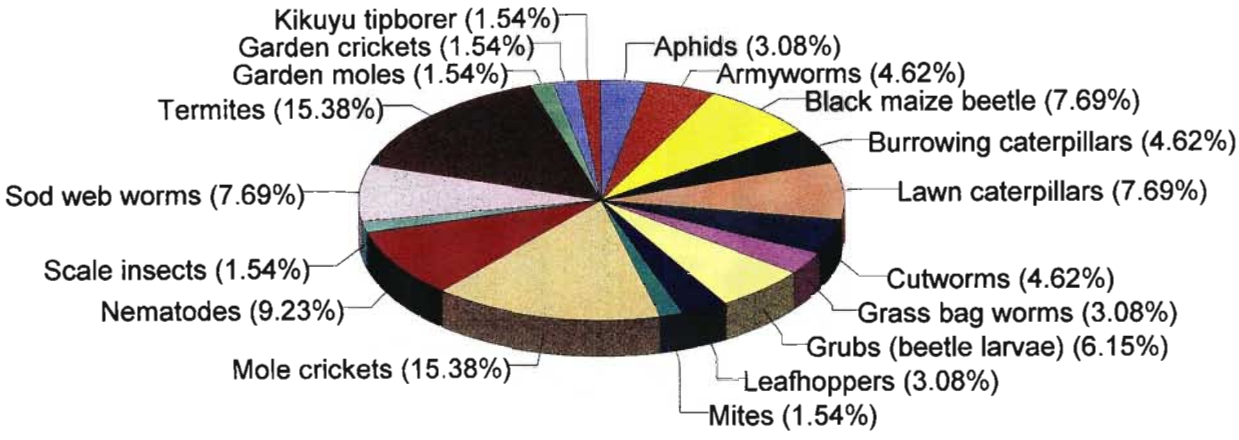


Figure 3.24 Insects and other pests commonly encountered by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Figure 3.25 summarises the insecticides used by the groundsmen included in the survey (Refer to Appendix 6 for a list of trade names and active ingredients of insecticides used). Of the responses received, 62% of the groundsmen did not disclose the insecticides or control measures implemented for the control of insect pests in turf.

The most commonly used insecticides identified in the survey, were Cyfluthrin, trade name sneak (23%) and Isofenphos, trade name peril (31%) for control of crickets and the burrowing and lawn caterpillars. Chlorpyrifos, trade name Dursban (15%) was identified for the control of cutworm. The pesticide Cypermethrin (trade name and active ingredient) (15%) was considered a general insecticide offering control of a number of insects shown in Figure 3.24.

In terms of alternates to chemical control, 23% of the groundsmen implemented cultural control and 8% (only 1 groundsman) used biological control.

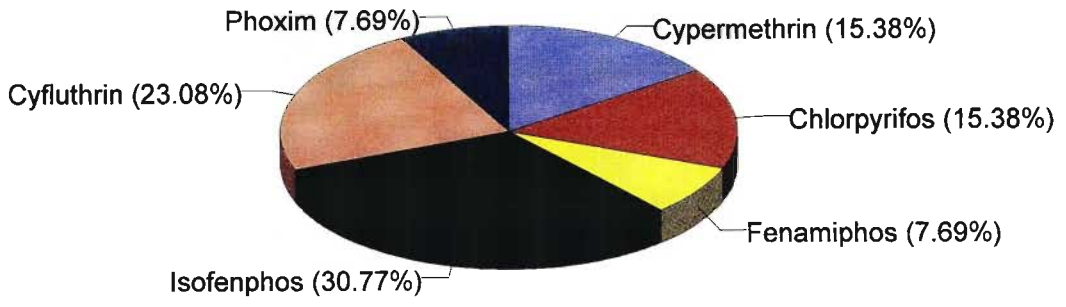


Figure 3.25 Insecticide use as identified by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

3.3.2.10 Disease management

Fungal diseases commonly encountered by the surveyed groundsmen are summarised in Figure 3.26.

Dollar spot (21%) and fairy rings (17%) were the most common disease in the survey area. Diseases such as brown spot, brown patch, turfgrass rust, Pythium blight and the leaf spot diseases (Cynodon and Kikuyu) (8% each) were also encountered. At present kikuyu yellows, a major disease of pasture grasses, accounted for only 6% of diseases commonly encountered.

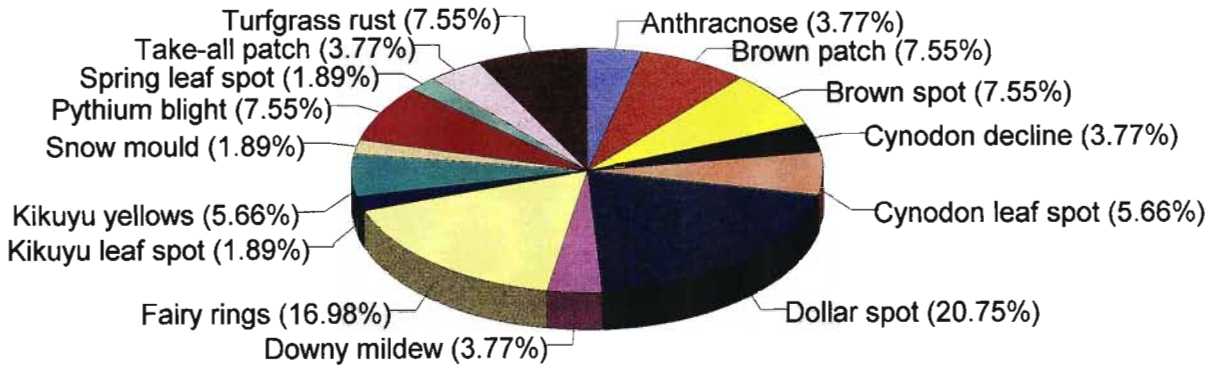


Figure 3.26 Turfgrass diseases commonly encountered by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Except for one groundsmen, disease control was largely attributed to fungicide use. Figure 3.27 summarises the fungicide active ingredients identified by the surveyed groundsmen.

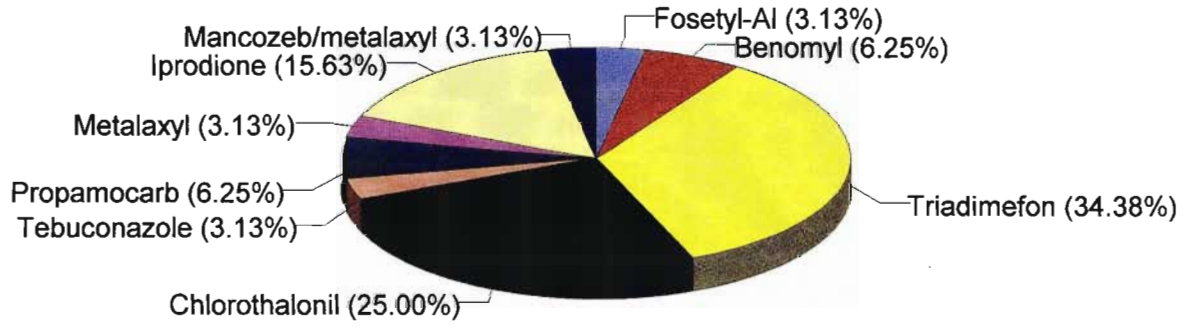


Figure 3.27 Fungicides identified for the control of turfgrass diseases encountered by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

Cultural practices are summarised in Figure 3.28. Topdressing (23%) was identified as the most commonly used method of cultural control.

Biological (8%) and integrated approaches (15%) to disease control were also practiced by some groundsmen.

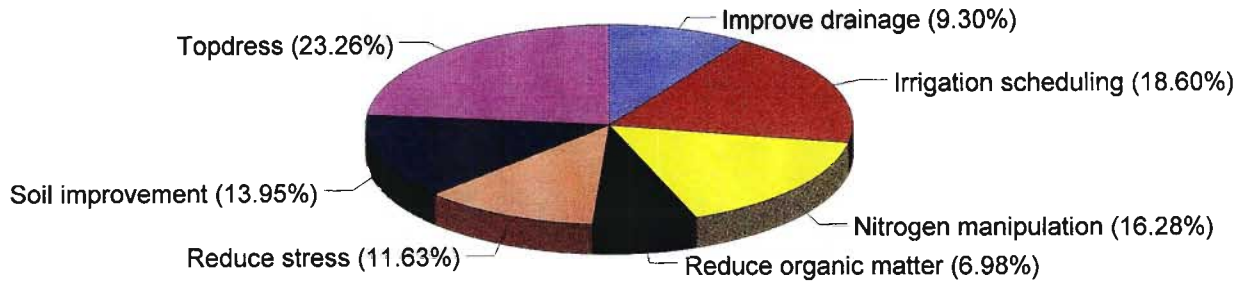


Figure 3.28 Use of cultural control measures for the control of turfgrass diseases commonly encountered by KwaZulu-Natal groundsmen. From a turf production survey (1999/2000).

3.3.2.11 Understanding of biological control and proposed use

Being a new concept to many, 31% of the surveyed KwaZulu-Natal groundsmen indicated that they had little to no understanding of biological control. However, 46% indicated that they had a clear understanding of the topic and would implement biological control if given the option. Major concerns expressed by the groundsmen were the cost, efficiency and safety issues.

3.3.3 RESULTS OF STATISTICAL ANALYSIS

There was very little significance in terms of the effect of management practices on pest incidences. Only the significant ($P \leq 0.1$) results have been recorded.

A number of these significant results are, however, unstable due to the expected frequency being less than five, as is expected for one degree of freedom (df) (Rayner, 1969).

3.3.3.1. Pasture production survey

Table 3.6 summarises the statistical analysis (Chi-square test) of the use of LAN (N fertilizer) on the occurrence of nutgrass (*Cyperus* spp.). The test revealed that the application of LAN was significantly ($P \leq 0.05$) associated with increased nutgrass occurrence.

Table 3.6. Summary of a Chi-square (χ^2) test to determine the influence of LAN on the occurrence of the commonly occurring weed, nutgrass (*Cyperus* spp.) as identified by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)

	Total nutgrass occurrences reported	%[1] nutgrass occurrence as per LAN use		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
Nutgrass	44	34	66	5.08	1	0.024 *

1 Percentage expressed as nutgrass occurrence with LAN utilization over the total nutgrass occurrences reported

2 Percentage nutgrass occurrence recorded by the farmers when LAN is not applied to the pasture as an N source

3 Percentage nutgrass occurrence recorded by the farmers when LAN is applied to the pasture as an N source

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

In terms of disease incidences, Table 3.7 summarises the results of a Chi-square test for the use of ammonium sulphate (N fertilizer) and disease occurrence. The test revealed that the application of ammonium sulphate was significantly associated with a reduction in the occurrence of the pasture diseases, ergot ($P \leq 0.05$), kikuyu yellows ($P \leq 0.1$), leaf blight ($P \leq 0.05$), ryegrass toxicity ($P \leq 0.05$) and tar spot ($P \leq 0.05$).

Table 3.7 Summary of a Chi-square (χ^2) test to determine the influence of ammonium sulphate $(NH_4)_2SO_4$ on the occurrence of pasture diseases, as identified by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)

Diseases* encountered	Total responses received for the diseases encountered	%[1] disease occurrence as per $(NH_4)_2SO_4$ utilization		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
Ergot	5	60	40	6.68	1	0.010** [a]
Kikuyu yellows	36	86	14	3.2	1	0.074* [a]
Leaf blight	7	71	29	3.91	1	0.048* [a]
Ryegrass toxicity	6	67	33	5.06	1	0.025* [a]
Tar spot	5	60	40	6.68	1	0.010** [a]

1 Percentage expressed as disease occurrence with $(NH_4)_2SO_4$ utilization over the total diseases encountered recorded

2 Percentage disease occurrence recorded by the farmers when $(NH_4)_2SO_4$ is not applied to the pasture as an N source

3 Percentage disease occurrence recorded by the farmers when $(NH_4)_2SO_4$ is applied to the pasture as an N source

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Significant results were also found for pest (weed, insect and disease) incidences associated with grazing management. This included grazing programs (Table 3.8) and the use of different grazing classes, a loaf camp and clean ups (Table 3.9).

Table 3.8 Summary of a Chi-square (χ^2) test to determine the influence of grazing programs on the occurrence of pests (weeds, insects and/or diseases), as identified by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)

Pests encountered*		Total responses received for the pests encountered	%[1] pest occurrence as per grazing programs		(χ^2) value	df[4]	Probability[5]
			(0)[2]	(1)[3]			
Grazing program - Blocks							
Weeds	Couch	28	68	32	4.03	1	0.045*
Insects	Armyworms	32	66	34	3.71	1	0.054*
Diseases	Kikuyu yellows	36	42	58	6.37	1	0.012**
	Leaf blights	7	14	86	5.17	1	0.023* [a]
Grazing program - Strips							
Weeds	Broadleaves	55	33	67	3.83	1	0.050* [a]
	Couch	28	18	82	7.17	1	0.007**
Insects	Birds	23	22	78	3.02	1	0.082*
	Ladybirds	17	18	82	3.24	1	0.072*
	Locusts	18	17	83	3.92	1	0.048*
Diseases	Kikuyu yellows	36	47	53	6.32	1	0.012**

1 Percentage pest occurrence as determined by the grazing program over the total pest occurrence recorded

2 Percentage pest occurrence recorded by the farmers when the grazing program is not implemented on the pasture

3 Percentage pest occurrence recorded by the farmers when the grazing program is implemented on the pasture

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Table 3.9 **Summary of a Chi-square (χ^2) test to determine the influence of grazing management on pest occurrence (weeds, insects and diseases), as identified by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)**

Pests* encountered		Total responses received for the pests encountered	%[1] pest occurrence as per management		(χ^2) value	df[4]	Probability[5]
			(0)[2]	(1)[3]			
1. Use of different class animals							
Insects	Ground weevils	16	69	31	2.84	1	0.092*
Diseases	Choke	3	100	0	3.06	1	0.080* [a]
	Kikuyu yellows	36	39	61	5.62	1	0.018**
3. Use of a loaf camp							
Insects	Buck	5	20	80	7.32	1	0.007** [a]
Diseases	Kikuyu yellows	36	64	36	3.13	1	0.077*
4. Use of clean ups note: only four farmers do not clean up making χ^2 unstable							
Insects	Bollworms	21	14	86	2.69	1	0.100* [a]
	Moles	3	33	67	3.36	1	0.067* [a]

1 Percentage pest occurrence as determined by the management practices over the total pest occurrence recorded

2 Percentage pest occurrence recorded by farmers when management practices are not implemented on the pasture

3 Percentage pest occurrence recorded by farmers when management practices are implemented on the pasture

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Use of grazing blocks was significantly associated with a reduction in the occurrence of the weed, couch grass (*C. dactylon* (L.) Pers) ($P \leq 0.05$). Use of grazing strips significantly increased the occurrence of couch grass ($P \leq 0.05$), as well as broadleaf weeds ($P \leq 0.05$). This was similar for insect pests, where grazing blocks were associated with lower incidences of armyworm ($P \leq 0.1$). Grazing strips were associated with a higher incidences of locusts ($P \leq 0.05$), ladybirds ($P \leq 0.1$) and birds ($P \leq 0.1$). The occurrence of kikuyu yellows was significantly decreased with both block and strip grazing ($P \leq 0.05$). Grazing blocks were also associated with a lower incidence of leaf blight diseases ($P \leq 0.05$).

Weed incidences were not affected by the grazing factors listed in Table 3.2, but these did significantly impact on the occurrence of insects and diseases, as summarised in Table 3.9. Increased incidences of the pests, buck ($P \leq 0.05$), bollworms ($P \leq 0.1$) and moles ($P \leq 0.1$), were associated with the use of a loaf camp and clean ups. Use of different grazing classes significantly decreased the occurrence of ground weevils ($P \leq 0.1$). The occurrence of kikuyu yellows was significantly increased by using different grazing classes ($P \leq 0.05$), but was lower when a loaf camp was included in the grazing rotation ($P \leq 0.1$). The chi-square test revealed that different grazing classes offer complete control of choke disease ($P \leq 0.1$).

Table 3.10 summarises the statistical analysis (Chi-square test) of the pests encountered and control measures implemented by the KwaZulu-Natal Midlands farmers. In terms of weed control, with increased incidences of broadleaf weeds a significant ($P \leq 0.05$) increase in the use of the herbicide 2,4-D occurred. This was also noted for increased incidences of invaders grass species (kikuyu) and nutgrass, but 2,4-D is effective only against broadleaf weeds (See Appendix 5). Significant control of broadleaf weeds was also achieved with the use of Garlon ($P \leq 0.05$) and physical weeding ($P \leq 0.1$). Control of nutgrass was significant with the use of MCPA ($P \leq 0.05$) and chopper ($P \leq 0.1$), which offered complete control. Roundup accounted for significant ($P \leq 0.1$) control of kikuyu, while physical labour (a cultural practice) significantly ($P \leq 0.1$) decreased annual grass populations. In terms of insect control using insecticides, the use of Karate significantly controlled armyworm ($P \leq 0.1$), caterpillars ($P \leq 0.05$) and cutworm ($P \leq 0.1$).

Table 3.10 Summary of a Chi-square (χ^2) test to determine the impact of control measures implemented for pest (weed and insect) control, as identified by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)

Weeds encountered	Total responses received for the weeds encountered	Control measure: Herbicide	%[1] weed occurrence as per control measure		(χ^2) value	df[4]	Probability[5]
			(0)[2]	(1)[3]			
Broadleaf	55	Garlon	93	7	4.40	1	0.036* [a]
		2,4-D	24	76	5.80	1	0.016**
Nutgrass	44	Dicamba MCPA	95	5	4.31	1	0.038* [a]
		2,4-D	16	84	10.78	1	0.001** [a]
		Chopper	100	0	3.45	1	0.063* [a]
Kikuyu	31	2,4-D	13	87	6.31	1	0.012**
		Round-up	90	10	2.66	1	0.103*
	Total responses received for the weeds encountered	**Control measure: Cultural**	**%[1] weed occurrence as per control measure**		**(χ^2) value**	**df[4]**	**Probability[5]**
			(0)[2]	**(1)[3]**			
Annual grasses	37	physical labour	68	32	2.75	1	0.097*
Broadleaf	55	physical labour	62	38	3.06	1	0.080* [a]
Insect pests encountered	**Total responses received for the insect pests encountered**	**Control measure: Insecticide**	**%[1] insect occurrence as per control measure**		**(χ^2) value**	**df[4]**	**Probability[5]**
			(0)[2]	**(1)[3]**			
Armyworm	32	karate	88	12	3.36	1	0.067* [a]
Caterpillars	26	karate	85	15	5.13	1	0.024* [a]
Cutworm	3	karate	67	33	3.36	1	0.067*

1 Percentage weed or insect occurrence as determined by control measures over the total pest occurrence recorded

2 Percentage pest occurrence recorded by the farmers without the implementation of a control measure

3 Percentage pest occurrence recorded by the farmers with the implementation of a control measure

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Regression analysis showed a significant ($P \leq 0.05$) positive simple linear regression for total weed control and total herbicides applied. The regression is

$$y = 0.510x + 2.821 \ (R^2 = 5.9; \text{ F probability} = 0.038 \text{ with } 55 \text{ df}) \quad (1)$$

Of the KwaZulu-Natal Midlands pasture farmers who encountered kikuyu yellows and rust, the Chi-square test results in Table 3.11, revealed a significant association ($P \leq 0.05$ for kikuyu yellows and $P \leq 0.1$ for rust) occurrence between the majority of the farmers who did not consult a plant pathologist for positive disease identification, but still indicated that kikuyu yellows and rust were prevalent on their farms.

The implementation of biological control revealed no significance. However, Table 3.12 shows significantly ($P \leq 0.1$) that the surveyed farmers, whether they have a good knowledge or no understanding of the concepts of biocontrol, would consider implementing biocontrol.

Table 3.11 Summary of a Chi-square (χ^2) test to determine the significance of consulting a pathologist for positive identification diseases encountered by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)

Diseases encountered	Total responses received for diseases encountered	%[1] disease occurrence as per pathologist consultation		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
Kikuyu yellows	36	69	31	5.31	1	0.021* [a]
Rust	26	69	31	2.72	1	0.099*

1 Percentage disease occurrence as determined by a pathologist over the total disease recorded

2 Percentage disease recorded by the farmers when a pathologist was not consulted for positive disease identification

3 Percentage disease recorded by the farmers when a pathologist was consulted for positive disease identification

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Table 3.12 Summary of a Chi-square (χ^2) test to determine the significant association between the use or potential use of biocontrol due to a preexisting knowledge of biocontrol, as identified by KwaZulu Natal Midlands farmers. From a pasture production survey (1999/2000)

Understanding of biocontrol	Total responses received for each level of understanding	%[1] use of biocontrol as per understanding		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
Clear understanding	13	8	92	5.45	2	0.066* [a]
Uncertain	6	17	83			
no knowledge	29	41	59			
	48					

1 Percentage use of biocontrol as determined by the levels of understanding over the total number of responses received for each level of understanding

2 Percentage of farmers rejecting biocontrol use based on their level of understanding

3 Percentage of farmers accepting/implementing biocontrol based on their level of understanding

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

3.3.3.2 Turf production survey

Due to the continuous variables, regression analysis was performed to determine the impact of management practices on pest incidences.

Regression analysis of the total number of pest occurrences noted (weed, insects and disease) and different topdressing depths (1-150mm) and frequency of topdressing (ranging from once per annum to every month), showed a significant ($P \leq 0.05$) positive simple linear regression for insect occurrence and increasing depth of topdressing. The regression is

$$y = 0.079x + 3.37 \ (R^2 = 27.7; \text{ F probability} = 0.046 \text{ with } 10 \text{ df}) \quad (2)$$

In terms of mowing height (2.3-20.9mm) and its potential effect on weed, insect and disease occurrences, regression analysis showed a significant ($P \leq 0.05$) negative simple linear regression for the total number of weeds encountered and increasing mowing heights. The regression is

$$y = (-0.983)x + 19.70 \ (R^2 = 29.1; \text{ F probability} = 0.05 \text{ with } 9 \text{ df}) \quad (3)$$

In terms of increased pesticide (herbicides, insecticides and fungicides) use associated with increased pest occurrences, regression analysis showed a significant ($P \leq 0.05$) positive simple linear regression for the total number of weeds encountered and increased use of herbicides. The regression is

$$y = 0.878x + 0.09 \ (R^2 = 36.2; \text{ F probability} = 0.018 \text{ with } 11 \text{ df}) \quad (4)$$

Table 3.13 summarises the statistical analysis (Chi-square test) used to determine the relationship between cultural control measures and the total number of diseases and insects encountered by the surveyed KwaZulu-Natal groundsmen. A significant ($P \leq 0.05$) relationship was found between those groundsmen who encountered a greater number of insects ($n \geq 14$) and implemented cultural control measures.

Table 3.13 Summary of a Chi-square (χ^2) test to determine the association between the total insect pests encountered and cultural control implemented, as identified by KwaZulu Natal groundsmen. From a turf production survey (1999/2000)

No. of insects encountered	Total responses received for insects encountered	%[1] of groundsmens who encountered insect pests as per the implementation of cultural practices		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
1	13	8	-	13	5	0.023* [a]
2		15	-			
3		23	-			
4		31	-			
5		8	-			
14		-	15			

1 Percentage of groundsmen to encounter insect pests as per the implementation of cultural control measures

2 Percentage of groundsmen to encounter insect pests without the influence of cultural control measures

3 Percentage of groundsmen to encounter insect pests with the influence of cultural control measures

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Of the cultural disease control practices outlined to the groundsmen (Figure 3.24), soil improvement practices were significantly ($P \leq 0.1$) associated with varying disease incidences. In general, soil improvement was associated with a greater number of diseases ($n \geq 9$) encountered by individual groundsmen (Table 3.14).

Table 3.14 Summary of a Chi-square (χ^2) test to determine the effect of soil structure improvement on the total number of diseases encountered, as identified by KwaZulu Natal groundsmen. From a turf production survey (1999/2000)

No. of diseases encountered	Total responses received for diseases encountered	%[1] of groundsmen who encountered diseases as per the implementation of soil improvement		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
2	13	-	23	10.99	5	0.052* [a]
3		23	-			
4		8	8			
5		23	-			
9		-	8			
15		-	8			

1 Percentage of groundsmen to encounter diseases as per the implementation of soil improvement

2 Percentage of groundsmen to encounter diseases without the influence of soil improvement

3 Percentage of groundsmen to encounter diseases with the influence of soil improvement

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

Once again, biological control revealed no significance in terms of use. However, Table 3.15 shows significantly ($P \leq 0.05$) that of the surveyed groundsmen, those with a clear understanding of the concepts of biocontrol would implement it.

Table 3.15 Summary of a Chi-square (χ^2) test to determine the significant association between the use or potential use of biocontrol due to a preexisting knowledge of biocontrol, as identified by KwaZulu Natal groundsmen. From a turf production survey (1999/2000)

Understanding of biocontrol	Total number of groundsmen who have an understanding of biocontrol	%[1] use of biocontrol as per understanding		(χ^2) value	df[4]	Probability[5]
		(0)[2]	(1)[3]			
Clear	6	0	100	13	1	< 0.001* [a]

1 Percentage use of biocontrol as determined by understanding biocontrol over the total number of groundsmen who understand the concept of biocontrol

2 Percentage of groundsmen rejecting biocontrol use based on their level of understanding

3 Percentage of fgroundsmen accepting/implementing biocontrol based on their level of understanding

4 Degrees of freedom

5 Significant below the 10% level ($P \leq 0.1$)

a Cells contain expected values less than 5 making the χ^2 test unstable

3.4 DISCUSSION

Pasture and turf surveys will be discussed separately, although there are many underlying management principles which are the same. Response to biological control will also be discussed separately, once management and pest incidences and control have been addressed.

Pasture survey

A sample size of 57 responses was received for the pasture survey. This was sufficient to draw viable conclusions. However, not all questions were addressed in the survey.

Soil type (Figure 3.1) impacts on pasture potential (Bartholomew 2000a) and the grass species cultivated (Bartholomew, 1991a). No single pasture species is able to maintain high growth rates, sustaining a year round grazing system. Therefore, pasture grasses are often mixed in specified ratios with legumes or other grass species. This increases palatability, digestibility and nutritional value, as well as potential utilization. It is for this reason that clover, oats, lucerne and the less commonly encountered pasture grasses were included in the survey. The value of clover mixes was emphasized by a survey response of approximately 10% utilization (Figure 3.2). However, South African farmers have been known to favour N fertilization of a pure pasture stand rather than the use of a legume-grass pasture (Donaldson, 2001).

Figure 3.3 confirms that the majority of KwaZulu-Natal Midlands farmers irrigate to supplement inadequate rainfall. As pastures are not a cash crop, an expensive sprinkler system is not warranted. The majority of farmers therefore used the non-mechanized sprinkler systems. The 3% of farmers using centre pivots may do so because they grow cash crops too.

The majority of farmers (77%) realized the importance of ensuring the efficiency of an irrigation system, as they consulted an irrigation specialist (Table 3.1). Land slope was given little consideration, although it has a direct impact on infiltration due to increased

run-off with steeper slopes. However, pasture establishment is predominantly achieved mechanically requiring flat land (Bartholomew, 2000a).

Acid soils are a major constraint in pasture production, especially in the KwaZulu-Natal Midlands (Miles and Manson, 2000). Of the surveyed farmers, 89% indicated the importance of prior soil analysis, with 22% of the total fertilizer amendments being attributed to lime, predominately dolomitic lime (Figure 3.4). Varying degrees of soil acidity tolerance are expressed by tropical and legume pastures (Reusch and Bennet, 2001). One would therefore, have expected greater utilization of tropical and legume pastures in the Midlands. However, legumes attributed only a small percentage of pasture species used (Figure 3.3).

Although the P and K status of soils, within the summer rainfall areas, is satisfactory, incorporation into the topsoil at planting is recommended (Drewes, 2000). Of the P sources presented to the farmers in the survey, 53% of the total responses recorded were attributed to Diammonium phosphate. Diammonium phosphate, monoammonium phosphate and superphosphate comprise a "readily available" P source, which is highly recommended for high producing perennial pastures (Miles and Bartholomew, 1987; Miles and Manson, 2000). Of the surveyed farmers, 88% indicated that they use potassium chloride as a K source. This is the cheapest source of inorganic K (Miles and Manson, 2000). Urea accounted for over half of the N fertilizers used by the surveyed farmers. This should be applied as a split topdressing to reduce losses due to mineralization or nitrification (Bartholomew, 2000a). Of the surveyed farmers, 91% indicated the advantages of split fertilizer applications. Excessive fertilizer applications, especially that of N, is often attributed to an increased incidence of pests (Couch, 1995). The application of LAN, accounting for 12% of fertilizers applied, was significantly ($P \leq 0.05$) associated with increased incidence of nutgrass. A higher potential for denitrification, is associated with LAN use, especially on poorly drained clay soils, such as was common to a number of the surveyed farmers as indicate in Figure 3.1. Denitrification decreases N availability to grass plants, reducing their potential to outcompete weeds. Weeds are also known to respond rapidly to improved soil fertility,

especially that of N (Medd *et al.*, 1987). Another N-source utilized was ammonium sulphate, but this accounted for only 2% of fertilizers applied. The significant decrease in pasture diseases associated with the application of ammonium sulphate may be attributed to improved plant health. This was true for the occurrence of ergot ($P \leq 0.05$).

The onset of spring is associated with the spread of the disease. Nitrogen-fertilization is recommended at this time, as active plant growth resumes. Plants which are N-deficient appear chlorotic. The occurrence of kikuyu yellows, leaf blights, ryegrass toxicity and tar spot are diseases associated with the appearance of chlorotic patches in spring. Often these disease symptoms may not be due to the pathogen but rather deficiency of an essential nutrient.

Fertilizer mixes/blends are often applied at establishment with "straight" fertilizers applied as a topdressing or in the case of severe deficiencies (Miles and Manson, 2000). Mixing fertilizers at different ratios is a common practice, with approximately 5% of pasture fertilization being dedicated to mixes (Figure 3.4). Zinc (Zn) deficiencies are common in the KwaZulu-Natal Midlands, and 2% of the total response for mixes was attributed to N:P:K:Zn.

Three farmers confirmed the use of chicken litter and farmyard manure. One of these responses was from an organic farmer. There are a number of consequences associated with unsterilized manure (Evans and Johnson, 1995). Variability in mineral balance and moisture content are further concerns (Meissner, 2000).

KwaZulu Natal Midlands farmers prefer rotational grazing. Rotational grazing incorporates efficiency in terms of irrigation and fertilization, but is labour intensive and time consuming (Bartholomew, 1991b). Rotational grazing was associated with better management and was significantly associated with reduced pest populations (Table 3.8). This was emphasized by farmers who implement grazing for cultural pest control (Figure 3.7). Grazing blocks accounted for the greatest reduction in weeds, insects and diseases. The alternate, strip grazing, is a more intensive program than rotational blocks, due to

the much reduced areas. Strip grazing requires the continuous forward movement of animals. The presence of pests is therefore more noticeable than in grazing blocks, where the animals move out to new blocks often some distance away. The decreased incidence of pasture diseases, kikuyu yellows and blight diseases, both expressing foliar symptoms was significant ($P \leq 0.05$) due to defoliation and regrowth of symptom-free foliage.

Questions asked about grazing management (Table 3.2) relate to the objectives of any grazing system (Edwards, 1987; Bartholomew, 2000b). Animals differ in terms of their daily dry matter requirements and consequently their grazing habits, and thus different animal classes should graze the pasture at any one time. The use of different classes was, however, not emphasized by farmers. For those who did use different grazing classes, a significant ($P \leq 0.1$) reduction in ground weevils was noted. This may be due to the pasture being grazed much lower, with insects being ingested. Due to the increased amount of plant material removed from the pasture, deficiencies are more pronounced. This may account ($P \leq 0.05$) the increase in yellowing (proposed kikuyu yellows) of pastures, noted in Table 3.9. Control of choke disease ($P \leq 0.05$) by means of grazing with different classes, may be accounted for by continuous grazing which reduces seed head (flower) formation.

Perennial pastures should not be allowed to flower (Edwards, 1987). This was agreed upon by 67% of surveyed farmers. New growth is generally more nutritious and palatable and flowering detracts from this (Donaldson, 2001). A pasture should also therefore, never be allowed to become stemmy. Mowing, as a clean-up, will maintain pasture quality. New growth after mowing (cleaning the pasture) may significantly ($P \leq 0.1$) have accounted for increased pest occurrences, especially bollworm as these feed on new growth (Table 3.9). Only 30% of the surveyed farmers indicated that they make use of a loaf camp. A loaf camp is included in rotational resting, for animal monitoring, supplementation with licks and general inoculations or dipping. In dairy production, cows are often placed into a loaf camp before and after milking. A loaf camp is also included for general pasture maintenance. This "recuperation" period may account for the

reduction ($P \leq 0.05$) in pasture yellowing (proposed kikuyu yellows). The significant ($P \leq 0.05$) increase in the incidence of buck on pastures was due to the absence of cattle. Weeds decrease pasture potentials, decreasing productivity and even being toxic to livestock upon ingestion (Leach *et al.*, 1987). The most commonly encountered weeds identified were grass species and nutgrass/nutsedge (Figure 3.6). The impact of weeds is greatest in a pasture less than 9 weeks after planting. Here, physical labour would be the more effective means of weed control, as the grass is still too short to mow or graze. Weeds control prior to planting should be sufficient to ensure a good grass stand that will shade-out weed species (Campbell *et al.*, 1987). Weed control on an established pasture is achieved by a carefully managed grazing system (Medd *et al.,* 1987). This was confirmed in the survey (Figure 3.7). On the whole, a 98% response was received for the use of cultural methods for weed control in comparison to the 88% response for herbicide usage. This may be attributed to the high costs associated with herbicides and labour, harmful chemical residues (Grobler *et al.,* 2000) and the implications associated with application, especially to an established pasture. Regression analysis (1) did, however, identify increased herbicide use with a higher weed occurrence ($P \leq 0.05$). Herbicide use may vary due to the types of weeds present, however, a non-selective herbicide will reduce all weeds at establishment. The increased use in herbicides also confirms the liberal use of herbicides as the ultimate means of weed control.

Although cultural weed control practices are more commonly used, herbicides do provide the most effective means of weed control, for the longest period of time (Campbell *et al.*, 1987). The majority of farmers indicated that they rely mostly on post-emergent weed control. In Figure 3.8, 2,4-D based herbicides and MCPA accounted for the greatest percentage of herbicides used by farmers. Both of these herbicides are registered for broadleaf weed control in established grass pastures (Grobler *et al.*, 2000). There has, however, been much debate on the use of 2,4-D and MCPA, and banning these herbicides has been suggested due to their detrimental effects (Grobler *et al.,* 2000). Furthermore, the survey showed a significantly ($P \leq 0.05$) higher use of 2,4-D with an increase of nutgrass, kikuyu and broadleaved weed incidences (Table 3.10). Farmers must be made aware of what herbicides are registered for the control of specific weed

types (refer to Appendix 5), in that 2,4-D is a selective herbicide registered for the use of broadleaf weeds. Significant control of broadleaves in the survey, was associated with Garlon ($P \leq 0.05$) and physical weeding ($P \leq 0.1$). Physical weeding is most efficient just after grass emergence where broadleaf weeds are easily identified and removed. Significant ($P \leq 0.1$) control of annual grasses (as a weed) by means of physical weeding was unexplained, as it would be difficult to identify which was planted and which was a weed, in this instance.

Chopper is registered for the control of certain species of noxious alien plants (woody species). Significant control ($P \leq 0.1$) of nutgrass was associated with the use of chopper. However, farmers should not use chopper on their pastures unless controlling bush encroachment or bugweed. Nutgrass, which was identified as a major weed of the KwaZulu-Natal Midlands (Figure 3.6), was significantly ($P \leq 0.05$) controlled with the use of MCPA, which is registered for control of nutsedge (nutgrass). On a mixed legume/grass pasture there are no herbicides available for weed control in an established pasture. However, prior to establishment of the pasture, pre-emergent broadleaf weeds, perennial and annual grasses (such as kikuyu) and nutgrass can be controlled with round-up. Eptam offers control against annual grasses and nutgrass in lucerne (i.e. broadleaf) pastures. For control of bramble (a noxious weed), garlon as a foliar application is recommended. Use of chopper and/or round-up, which are non-selective, will kill any plant material and chopper is soil active, having the potential to result in large dead patches in the pasture. Garlon also moves in the soil, killing all broadleaves that it comes into contact with.

Insect control is also heavily reliant on chemicals. However, only 17 (30%) of the farmers implement insect control strategies, with 25% of these being attributed to insecticides. The low response for control measures may be attributed to grazing and trampling of pasture insect pests (Allen, 1987). However, only 2 farmers (4%) attributed low insect populations to cultural practices. Generally, insect damage is of little importance to an established pasture, not warranting the expense or the withholding periods associated with chemical control. Biological control offers an alternative to chemical control, but

again farmer's responses were small (4%). Of interest, one farmer identified the use of natural/biological control by means of encouraging bird activity rather than identifying the birds as pests. Cutlass contains *Bacillus thuringiensis* (Nel *et al.* 2003), a bacterium offering insect pest control. Farmers therefore inadvertently implemented biocontrol.

Although survey results suggested little urgency for the control of insects, a large number of pests were encountered by farmers (Figure 3.9). This may be attributed to damp conditions, characteristic of the KwaZulu-Natal Midlands, which are ideal for insect pests. The low response may also be attributed to the fact that insect pests feed at night and are therefore difficult to identify conclusively. Armyworm, although not considered severe, was the pest identified as most critical to control. The majority of insect pests identified by the surveyed farmers cause damage to germinating seed and foliage, reducing pasture productivity.

Survey responses indicate that disease occurrence was also of little significance, unless the disease has a pathological effect on grazers. Of the diseases presented in Figure 3.11, ryegrass toxicity, ergot and choke result in poisoning of livestock after ingestion (Davies *et al.*, 1996; Moore, 1966). Diseases identified as being most severe were kikuyu yellows, rust and seedling death (71% of diseases encountered). The high occurrence of kikuyu yellows and rust may not entirely be so. Any yellowing of a pasture is generally immediately assumed to be kikuyu yellows, when it is often as a result of poor drainage or nutrient uptake. The same principle applies to rust, where farmers attribute any leaf spots to rust, when there are an array of leaf spot diseases associated with grass species. Incorrect disease diagnosis was confirmed by the number of farmers who indicated outbreaks of kikuyu yellows ($P \leq 0.05$) and rust ($P \leq 0.1$) but did not consult a plant pathologist for positive identification. Ryegrass blast is a fairly new disease to the Midlands. Disease incidences are associated with warm, humid conditions (Smiley *et al.*, 1992), characteristic of the KwaZulu-Natal Midlands in summer. The incidence of disease should therefore have been high, but the survey revealed a low disease incidence. This too may be attributed to incorrect disease diagnosis.

Many farmers believe that grazing is a means of eliminating disease effectively. However, grazing is a common means of disease spread (Hall, 1992). From Table 3.3., it was again evident that control is heavily reliant on chemicals. Fungicides identified by farmers were systemic (Nel *et al.*, 1999), indicating that control measures were only implemented once the disease was established. Cultural methods of control are linked to management practices. Only five farmers (9%) indicated that they implement cultural control methods. A zero response was recorded for that of biological and integrated control. This can be attributed to the lack of practical knowledge available to South African farmers, although these terms are becoming more common to farmers.

Turf survey

Responses received for the turf survey were less than that of the pasture survey. The single responses received from the sportfield and race course manager, has little impact in terms of determining general management and pest incidences. Therefore, the discussion is based largely on golf courses, however, the number of responses received from greenkeepers was also too small to render any significance.

Golf greens are predominately sand based. However, 75% of the groundsmen indicated that they preferred local specifications, which incorporates organic matter (Allen, 1999). Soil texture of a turf area is also improved by topdressing (Stewart, 1980; Vengris and Torello, 1982; Emmons, 1996). Light topdressing (1-10mm) of sterilized sand was considered optimal by the surveyed groundsmen (Figure 3.12). Sterilization ensures a disease and weed-free topdressing and the texture of the sand improves existing soil structure. Regression analysis (2) identified a significant ($P \leq 0.05$) increase in insect incidences with increased topdressing depth. This may be accounted for due to raking/brushing that is required after heavier topdressings which will disturb insects present on or within the grass mat. New growth through a topdressing is more palatable to insects, hence the increased pest occurrence associated with topdressing. Pine bark and animal manure contain high percentages of organic matter and are recommended for lighter soils. Factors to consider, in terms of the frequency of topdressing, are outlined in Table 3.4. Only one response was received for the consideration of thatch and

irrigation in terms of when to topdress. Thatch is of particular importance, as topdressing encourages nutrient cycling and has the potential to reduce disease and pest incidences. The majority of responses were recorded for topdressing annually or twice per annum. From the groundsmen's comments in the turf survey, it was concluded that topdressing was most frequent in the summer and spring, when growth resumes.

A golf green must comprise of thin/fine bladed grass species, that forms an even mat over the soil surface. Figure 3.13 identifies these grasses as Country club (*Paspalum vaginatum)*, Couch grass (*Cynodon dactylon*), Bayview (*C. transvaalensis*), Bentgrass (*Agrostis stolonifera*) and Lm grass (*Dactylotenium australe*). The low percentage use of the latter three could be attributed to their use as a mixture with *C. dactylon. Paspalum vaginatum* is also commonly cultivated with *C. dactylon. Cynodon dactylon* is a warm season grass. Mixtures, therefore improve colour persistence, as well as producing a higher quality turf, with better establishment, an even surface cover and higher tolerance to wear, disease and shade (Schroeder and Sprague, 1996).

Predominant grasses used for establishment of golf tees and fairways, are *kikuyu* (*Pennisetum clandestinum*) and *Cynodon spp.* Mention was given to Buffalo grass (*Stenotaphrum secundatum*) for use on fairways, as it is considered to be a coarse, hardy stoloniferous perennial grass that forms a dense, bright green sward. Race courses and sportsfields also commonly comprise kikuyu (Cudney *et al.*, 1993), which forms a robust, dense and soft stand reducing potential injuries.

Cynodon dactylon and *P. vaginatum* (Figure 3.13) are two grasses that are predominately used in the establishment of golf greens, both requiring frequent irrigation (Turgeon, 1991; Schroeder and Sprague, 1996). This was confirmed by a 73% survey response for fixed irrigation on golf greens. The high irrigation requirement, is also due to the greens being predominately sand-based. Approximately 69% of the surveyed groundsmen indicated that they implemented irrigation scheduling (Figure 3.14). Simple visual observations and weather monitoring, include determining when the soil moisture is exhausted to the point that the grass wilts at midday. The efficiency of such a system

is determined by the level of management and the groundsmen's knowledge of plant physiology. Visual observations also allow for the early detection of pest outbreaks. Electronic scheduling, entailing an automated computer system, allows for a more controlled means of implementing irrigation, rather than relying on physically moving sprinklers/draglines. A tensiometer is useful in monitoring soil moisture (Reinders, 1992), and can be incorporated with both simple observations and electronic irrigation scheduling. An evaporation pan to determine irrigation requirements is considered inaccurate, even though it accounted for 17% of the survey responses. An evaporation pan only determines evaporation from a water source, not taking into account evapotranspiration.

Pop-ups are considered the easiest and most efficient irrigation system to implement on golf greens, race tracks and sportsfields as confirmed in Figure 3.15. The use of draglines and portable pipes, although more labour intensive, is also common on golf greens and tees. Sprinklers and springer heads, which are attached to draglines and pipes, are positioned higher from the soil surface than pop-ups. Droplet size and drift are therefore, much greater than that of pop-ups.

The majority of surveyed groundsmen chose to irrigate in the early morning. Irrigation should be to field capacity to compensate for transpiration losses during the day (Emmons, 1996). A high response was also received for midday irrigation. The advantage of doing this is to cool the plants, however, being the hottest time of day evaporation rates are high reducing efficiency. Late afternoon irrigation will reduce evaporation losses, but the plants will suffer moisture stress, although this is also largely determined by the frequency and depth of the previous irrigation. If irrigation is too late in the afternoon, leaf wetness is prolonged creating ideal conditions for the spread and development of a number of turf diseases.

Good surface and subsurface drainage is essential for good turf growth, ensuring a balance between moisture content and aeration of the soil (Escritt, 1980). Drainage can be either artificial or natural. Of the drainage systems presented in Figure 3.17, artificial

drains accounted for only 29% of drainage practices implemented. The blanket drain and sand drainage systems make use of the natural drainage properties of gravel and sand. These are generally cheaper and easier to implement, hence the higher response.

The importance of soil analysis cannot be stressed enough. All but one groundsman, indicated that they made use of soil sampling to maintain a sustainable soil. Once requirements have been determined, consideration must be given to the method of application. Granular fertilizers were most popular. These are predominantly applied to tees and fairways, while liquid fertilizers, such as N and P via fertigation (including boomsprayers), are applied to the greens. This stands to reason in that greens require more irrigation. Granular fertilizers and lime are best applied with a spreader (Figure 3.18) and then worked into the soil or followed by irrigation. A walk-behind spreader is suitable for smaller areas and a granular spreader, hopper or spandicar for larger areas. Hand applications were also noted, but coverage is not always even.

Incorrect mowing practices do more damage to turf than any other maintenance practices (Emmons, 1996). A reel mower is commonly used on golf greens, tees and fairways. The Toro 3200 was the more commonly used reel walk-behind mower (Figure 3.19), and can be used on sportsfields too. The Jacobson Greens King IV is a ride-on mower alternative, especially for expansive golf courses. A walk-behind mower is, however, of a greater advantage as a closer, more even cut is achieved (Tame, 1999).

It is not only the type of mower but also the frequency of mowing (Table 3.5) and the mowing heights (Figure 3.20) that are important in maintaining a high quality playing surface (Corbett, 1998). In summer when rainfall and temperatures are higher, grass growth will be more abundant requiring frequent mowing. Greens, in comparison to tees and fairways, require frequent mowing to maintain the short height required. Figure 3.20 summarises the average heights that should be maintained. If stress damage is noted, mowing height should be increased by 1-2mm (Tame, 1999). However, turf managers prefer to mow to the lowest possible height that can be tolerated, as too frequent mowing hampers regrowth resulting in the appearance of dead patches and weed invasions

(Emmons, 1996). Effective weed control was, however, significantly ($P \leq 0.05$) determined at a lower mowing height (3). However, the reduction in weed occurrence may simply be attributed to the removal of foliage. A lower mowing height will impact on the competitiveness of the weeds, in that weeds are continuously forced to direct energy to the formation of new leaves, without producing reproductive structures.

A turf grass that is cut too long will accumulate a thick thatch layer, decreasing soil aeration and growth (Schroeder and Sprague, 1996). Aeration/decompaction can be achieved by removing small cores, of which hollow tyning (which should also include coring) was identified as the more frequently used method (Figure 3.21). Hollow tyning can be implemented at any time during the season and is often done in conjunction with topdressing, filling the holes and improving soil structure. Verti-draining (15% response) is the quickest and easiest means of relieving decompaction.

All the above cultural practices emphasise the need for a uniform turf, however, weed incidences break this uniformity. Weed incidences varied for each groundsmen. This is largely attributed to management practices, prevailing environmental conditions and the grass species comprising the turf stand (Emmons, 1996). The most commonly encountered weed was clover (Figure 3.22). In a turf stand the trifoliate appearance of the clover breaks uniformity, especially when in flower. Clovers are more vigorous than turf species, out-competing turf species. This vigour is also expressed by grass invaders and watergrass, also identified as commonly encountered weeds. Control of weeds was predominately by means of herbicides (Figure 3.23), with a significant ($P \leq 0.05$) increase in the number of herbicides applied according to the number of weeds encountered (4). Of the herbicides identified, glyphosate (trade name: round-up and sting) was the more frequently used active ingredient. With the controversy surrounding methyl bromide (De Ceuster and Hoitink, 1999), glyphosate would be the first choice for non-selective pre-emergent weed control. Non-selective, post-emergent herbicides, such as MSMA, offers effective control against annual grass invaders, clover and other broad-leafed weeds, as well as watergrass. Although herbicides offer effective control, benefits are only temporary and should therefore be accompanied by a good maintenance program. This

includes mowing and hand weeding when weed populations are manageable.

Where pest and disease incidences were considered not important in pasture production, they are of major concern to groundsmen, with all of survey responses indicating insect pest occurrences. Caterpillars were the most commonly encountered insect pest in the survey area (Figure 3.24). Caterpillars feed predominately at night or under the foliage mat and are only detected once damage has occurred. Crickets also were commonly encountered causing damage to the turf and roots. Crickets, as well as cutworms, termites and grubs result in a turf that is easily dislodged. The burrows of termites and crickets often result in an uneven playing surface as they under-mine the turf.

The majority of above mentioned pests are controlled by insecticides containing the active ingredient, Cypermethrin. The most commonly used active ingredients identified in the survey, were Cyfluthrin (sneak) and Isofenphos (peril) for the control of root-feeding insects (Figure 3.15). The advantage of sneak is that it can be applied either as a preventative or curative spray. It is vital that the pesticide used is registered for the pest. For example, the active ingredient of dursban is chlorpyrifos, identified in the survey for the control of termites. However, according to Nel *et al.* (2002), the registered active ingredient for termite control is Permethrin. Another discrepancy was that of Phoxim. This was identified in the survey for control of moles, but is is registered for control of ants and storage insects (Nel *et al.,* 2002).

Of the responses received, 62% of the groundsmen did not disclose chemicals that they used. Of this 62%, the majority of groundsmen encountered severe outbreaks of one or more pests. The lack of response may be attributed to the controversies surrounding chemical use (refer to Chapter 1), alternative control measures are thus important. However, low responses were received for the use of cultural and biological control. Groundsmen who did use cultural control methods, encountered higher insect pest incidences ($P \leq 0.05$). Insect occurrence would be expected to be higher for groundsmen relying solely on cultural measures of control, an integrated approach with both chemical and cultural practices should provide better control.

Response to the control of diseases using fungicides differed to that of insect control with 92% of the groundsmen indicating chemical use. Bounce turfgrass fungicide (active ingredient Triadimefon) was identified as the most commonly used fungicide, with 75% of the groundsmen indicating that they rely on more than one fungicide for disease control (Figure 3.27).

Disease control was also attributed to the prevention of conditions that are conducive to disease development (Figure 3.28). However, a significant ($P \leq 0.1$) association between high disease incidence and implementation of cultural control measures was noted, in that groundsmen who initiated soil structure improvement encountered diseases. Soil improvement often entails coring, topdressing or complete reconstruction of the turf area to create ideal growth conditions. The Chi-square test preformed here was unstable due to the expected values, hence perhaps the unexplainable result.

Disease occurrence is also largely predetermined by the grass species present. There is limited information on which turfgrass cultivars are more prone to which diseases under South African conditions, and most greenkeepers have established their knowledge from trial and error experiences. Other than the use of resistant cultivars, biological and integrated approaches to disease control were also noted. These control measures are going to impact greatly on disease control in the future, but are at present fairly unknown, hence the low survey response.

Disease occurrence ranged from only one recorded disease to 11 disease outbreaks. Common to a closely mowed turf is dollar spot (Couch, 1995). Visually this disease is easy to identify and was identified by the groundsmen as the most commonly encountered disease. Many groundsmen also regard fairy rings as severe, in that control measures are erratic and costly. Diseases such as brown spot, brown patch, turfgrass rust, Pythium blight and the leaf spot diseases were also encountered. These diseases all have the potential to kill infected turfgrass, however, once detected effective control measures are available (refer to Chapter 2). The low response for kikuyu yellows in turf may be attributed to close mowing (i.e. frequent defoliation).

Response to proposed biological control

The future of biocontrol is reliant on public perception. Farmer's responses as to whether they would consider using biocontrol was 61% for implementation, even though almost 51% indicated that they had no knowledge of biological control. It can, therefore be assumed that farmers would be "open" to the use of biocontrol. Only one farmer indicated that at present biological control is not applicable to pastures. Similar positive responses to the implementation of biocontrol were received from 46% of KwaZulu Natal groundsmen. The number of farmers and groundsmen who understood the concept of biocontrol and were willing to implement such a measure was significant (Tables 3.12 and 3.15). This emphasizes the need to educate more farmers and groundsmen about the potential of biocontrol. Major concerns expressed by groundsmen and farmers were cost, effectiveness and safety of biocontrol agents. If these concerns are addressed, the potential for biocontrol is promising. This was confirmed by the farmers and groundsmen identifying the need for ongoing research for chemical alternatives. One groundsmen even went as far as adding that if nature is able to control diseases in its own way, then why work against it.

3.5 REFERENCES

Allen, P.G. 1987. Insect pests of pastures in perspective. In: J.L. Wheeler, C.J. Pearson and G.E. Robards (eds). Temperate pastures: their production, use and management. Australian Wool Corporation/CSIRO, New South Wales: Australia. p. 217-218.

Allen, S. 1999. By trial and error: turfgrass varieties for the golf course. In: C. Knoll (ed). Golf clubs and associations of Southern Africa, Sun City Conference, 11-14 July 1999. Turf & Landscape Maintenance, October/November 1999:15.

Anon, 2000. Genstat for Windows. Release 4.2, 5th edition. VSN International Ltd, Oxford: United Kingdom.

Bartholomew, P.E. 1991a. Adaption of pasture species. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Bartholomew, P.E. 1991b. Pasture utilization: grazing systems. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Bartholomew, P.E. 2000a. Establishment of pastures. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 156-159,163.

Bartholomew, P.E. 2000b. The management of planted pastures: humid regions, summer rainfall region. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 233-255.

Campbell, M.H., W.J. Hosking, D.A. Nicholas, E.D. Higgs and J.W. Read. 1987. Establishment of perennial pastures. In: J.L. Wheeler, C.J. Pearson and G.E. Robards (eds). Temperate pastures: their production, use and management. Australian Wool Corporation/CSIRO, New South Wales: Australia. p. 59 -74.

Corbett, G. 1998. Management techniques affecting ball roll speed. In: K. Richards (ed). Turf & Landscape Maintenance, February/March 1998.

Couch, H.B. 1995. Diseases of turfgrasses, 3rd edition. Krieger Publishing, Florida: United States of America.

Cudney, D.W., J.A. Downer; V.A. Gibeault; J.M. Henry and J.S. Reints. 1993. Kikuyu grass (*Pennisetum clandestinum*) management in turf. Weed Technology **7:** 180-184.

Davies, S.C., C.L. White, I.H. Williams, J.G. Allen and K.P. Croker. 1996. Sublethal exposure to corynetoxins affects production of grazing sheep. Australian Journal of Experimental Agriculture **36:** 649-655.

De Ceuster, T.J.J. and H.A.J. Hoitink. 1999. Using compost to control plant diseases. BioCycle, June 1999. p. 61-64.

Donaldson, C.H. 2001. A practical guide to planted pastures. Kalbas Publishers, Cape Town: South Africa. p. 93-101.

Drewes, R.H. 2000. Establishment of pastures: semi-arid regions. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 173.

Edwards, P.J. 1987. Grazing managment. In: Pasture production manual. Directorate of Agricultural Information, Department of Agriculture and Water Supply, Pretoria: South Africa.

Emmons, R. D. 1996. Turfgrass science and management, 2nd edition. Delmar Publishers, New York: United States of America.

Escritt, J.R. 1980. Construction and maintenance of sports turf. In I.H. Rorison and R. Hunt (eds). Amenity grassland, an ecological perspective. John Wiley & Sons, Salisbury: United Kingdom. p. 125-135.

Evans, J.A.H. and P.T.C. Johnson. 1995. Concentrates for dairy cattle. In: M. Abbott (ed). Dairying in KwaZulu-Natal.Co-ordinated Extension Committee of KwaZulu-Natal, KwaZulu-Natal Department of Agriculture, Pietermaritzburg: South Africa. p. 166.

Grobler, H., J.B. Vermeulen and K. van Zyl. 2000. A guide to the use of herbicides, 17th edition. National Department of Agriculture, Pretoria: South Africa.

Hall, A.S. 1992. Pasture grass diseases. In: Trench, T.N., D.J. Wilkinson and S.P. Esterhuysen (eds). South African plant disease control handbook: Farmer Support Group. Kendall & Strachan, Pietermaritzburg: South Africa. p. 365-369.

Keeton, W.T. 1976. Biological science, 3rd edition. W.W. Norton & Company, Toronto: United States of America. p. 913.

Leach, G.J., R.M. Jones and R.J. Jones. 1987. The agronomy and ecology of improved pastures. In: N.H. Shaw and W.W. Bryan (eds). Tropical pasture research: principles and methods. Commonwealth Agricultural Bureuax, Farnham Royal: United Kingdom. p. 296-298.

Nel, A., M. Krause and N. Khelawanlall. 2002. A guide for the control of plant pests, 39th edition. National Department of Agriculture, Pretoria: South Africa.

Nel, A., M. Krause, N. Ramautar and K. van Zyl. 1999. A guide for the control of plant diseases. National Department of Agriculture, Pretoria: South Africa.

Medd, R.W., D.R. Kemp and B.A. Auld. 1987. Management of weeds in perennial pastures. In: J.L. Wheeler, C.J. Pearson and G.E. Robards (eds). Temperate pastures: their production, use and management. Australian Wool Corporation/CSIRO, New South Wales: Australia. p. 253 -265.

Meissner, H.H. 2000. Nutrient supplementation of the grazing animal. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 100-101.

Miles, N. and P.E. Bartholomew. 1987. Lime and fertilizer requirements of pastures. In: Pasture production manual. Directorate of Agricultural Information, Department of Agriculture and Water Supply, Pretoria: South Africa.

Miles, N. and A.D. Manson. 2000. Nutrition of planted pastures. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 208-216.

Moore, I. 1966. Grass and grasslands. Collins, London: United Kingdom. p. 79-80.

Rayner, A.A. 1969. A first course in biometry for agricultural students. University of Natal Press, Pietermaritzburg: South Africa.

Reinders, F.B. 1992. Irrigation systems. In: K.O. Bang (ed). Agricultural production guidelines for Natal: Irrigation in Natal. Department of Agricultural Development, Pietermaritzburg: South Africa.

Reusch, J.D.H. and R.J. Bennet. 2001. Know your cultivars! Perennial and hybrid ryegrass and tall fescue, the old and the new! Temperate forages and their role in livestock production: Agricultural Farmers Day 29th and 30th August 2001, Pietermaritzburg: South Africa.

Schroeder, C.B. and H.B. Sprague. 1996. Turf management handbook, 5th edition. Interstate Publishers, Illinois: United States of America. p. 5-147, 150-155.

Smiley, R.W.; P.H. Dernoeden and B.B Clarke. 1992. Compendium of turfgrass diseases, 2nd edition. American Phytopathology Society, Minnesota: United States of America.

Stewart, V.I. 1980. Soil drainage and soil moisture. In: I.H. Rorison and R. Hunt (eds). Amenity grassland, an ecological perspective. John Wiley & Sons, Salisbury: United Kingdom. p. 119-124.

Tame, J. 1999. The grow-in of Peaconwood golf course. Turf & Landscape Maintenance, April/May 1999: vol. 12.

Turgeon, A.J. 1991. Turfgrass management, 3rd edition. Prentice Hall, New Jersey: United States of America.

Vengris, J. and W.A. Torello. 1982. Lawns: basic factors, construction and maintenance of fine turf areas, 3rd edition. Thomson Publications, California: United States of America.

CHAPTER 4

POTENTIAL FOR THE BIOLOGICAL CONTROL OF HELMINTHOSPORIUM LEAF SPOT ON THE PANICOID GRASS USING AMENDED BIOCONTROL AGENTS

ABSTRACT

Biological control potential of antagonistic *Bacillus* and *Trichoderma* strains were evaluated against Helminthosporium leaf spot on *Pennisetum clandestinum* Chiov (kikuyu). *In vitro,* the observation of bipolar germination tubes confirmed *Bipolaris* sp. as the causal agent responsible for the disease found on kikuyu pastures at Cedara ($29^0$32'S, $30^0$17'E). Koch's postulate was proved. *In vitro* testing using an agar dual culture antagonism test, revealed *T. harzianum* Rifai T-22 to be the more virulent than *B. subtilis* Ehrenberg Cohn. Field trials were conducted in March-April 2000 and February-March 2002. Disease incidence in 2001 was not severe and in 2002, the trial site was moved due to a lack of disease incidence. Whether lower disease incidence was due to the application of the antagonists to the kikuyu pasture in the 2000 season was not conclusively established. The efficiency of the *Trichoderma* and *Bacillus*-based biocontrol formulations at half and full the manufacturer's recommended dose, was determined against the fungicide PUNCH XTRA® and a zero dose control treatment (i.e.,pure water). Potential disease control was determined by means of disease ratings over a 5 week period. The area under the disease progress curve was used to determine disease control associated with each treatment. Final percentage disease was also determined. *In vivo* field results were inconsistent and non-significant. However, results did confirm that the amended biocontrol treatments were effective in comparison to the control, i.e. no treatment. Against PUNCH XTRA®, disease control induced by the biocontrol agents was variable. The potential of *T. harzianum* as an alternative to

chemical disease control was suggested but not confirmed statistically. Disease control should offer increased plant growth, and thus plant biomass was also assessed.

4.1 INTRODUCTION

The concept of biological control is gaining interest as antagonistic potentials of microorganisms are being realized. Many chemical companies are now investing in the discovery and development of biological control agents (BCAs) (Froyd, 1997). Regeneration of organic farming principles, has also sparked an interest in biological control (Harman, 2000). This is especially true for integrated pest management, which provides an environment where the efficiency of BCAs can be easily determined (Williamson, 1998). Microorganisms that colonize the rhizosphere are ideal for use as BCAs (Weller, 1988). Specific *Bacillus* sp. (Baker *et al.*, 1985; Weller, 1988; Marrone, 1999) and *Trichoderma* sp. (Papavizas, 1985; Deacon and Berry, 1992; Lewis *et al.*, 1998; Harman, 2000) have been recorded to offer disease control and stimulated growth upon rhizosphere colonization. Both antagonists also have the potential to control foliar diseases (Blakeman, 1985; Agrios, 1997).

The aim of these trials was to determine the potential of *Bacillus* sp. and *Trichoderma harzianum* Rifai for control of Helminthosporium leaf spot, a common disease of *Pennisetum clandestinum* Chiov. (kikuyu). Kikuyu is a turf and pasture grass utilized in the KwaZulu-Natal Midlands. The disease detracts from the aesthetic value and production potential, thus control is necessary. Biological control agents offer a safer alternative to the liberal use of fungicides for disease control. Therefore, the efficiency of *T. harzianum* and *Bacillus* strains were compared to the fungicide PUNCH XTRA®, the active ingredient being carbendazim/flusilazole. The manufacturer's recommended dosage rates of the microbial formulations were also evaluated at half and full dose.

A further aim of the trial was to determine the taxonomy of the causal agent of Helminthosporium leaf spot in the KwaZulu-Natal Midlands. The appearance of disease symptoms may be attributed to four proposed pathogens *Drechslera*, *Exserohilum*, *Curvularia* and *Bipolaris* (Smiley *et al.*, 1992). Of these genera, the causal agent was isolated and determined by means of conidial classification, in terms of size, shape, colour and the development of germ tubes from the conidia.

4.2 MATERIALS AND METHODS

4.2.1 SCANNING ELECTRON MICROSCOPY (SEM)

Bipolaris infected leaf samples, on *P. clandestinum*, were collected from the 2000 trial site in the early morning to ensure cell turgidity. Single lesions, measuring approximately 3 mm x 3mm, were removed from the leaf material using a sterilized blade, and fixed in 3% gluteraldehyde in 0.05M sodium cacodylate buffer (pH 6.8-7.2) for 24hrs. The samples were washed twice in a buffer, post-fixed for two hrs in 2% osmium tetroxide in buffer, and dehydrated in a graded ethanol series. The specimens were then critical-point dried with carbon dioxide as a transfusion fluid. Dried specimens were mounted onto copper specimen stubbs, using colloidal graphite. On each stub, specimens were mounted at different angles i.e, abaxial side up, adaxial side up, cut edges up etc., to determine the best view of the fungal material. The leaf-fracture method of Hughes and Rijkenberg (1985) was used to examine hyphae within plant tissues. Specimens were sputter coated with a thin metal (gold:plalladium) layer in a Polaron Sputter Coater and viewed in a Hitachi S-570 scanning electron microscope operating at 8.0 and 10.0 kV.

4.2.2 *IN VITRO* TESTING

4.2.2.1 *Bipolaris* sp. isolation

Bipolaris sp. was isolated from *P. clandestinum* collected from the 2002 trial site. Single lesions were removed from the leaf material using a sterilized blade and surface sterilized in 1% Jik for two minutes, followed by two distilled water washes for the same time period. The abaxial surface of the serialized lesion was placed onto potato dextrose agar

and incubated at room temperature (approximately 23-25°C) for 72-96 hrs. Approximately 20 plates with four pieces of lesioned leaf material per agar plate were cultured. Upon the appearance of fungal hyphae growing into the agar, plates were re-cultured onto potato dextrose agar to eliminate contaminates to produce pure cultures. Cultures were then placed under alternating cycles of 12 hrs UV light and 12 hrs darkness for a period of 10 days to induce the formation of conidia (Zhang and Yeun, 1999). Spores were scrapped from the sporulating lesions, mounted in a drop of distilled water and viewed using a Zeiss light microscope (Zeiss, Germany) at 40X magnification to confirm the causal agent (Alcorn 1988; Smiley *et al.*, 1992). Spores were photographed using a Contax mountable camera 167MT SLR (Kyocera corporation, Japan).

4.2.2.2 Antagonism tests

Biological control potentials of *T. harzianum* and *B. subtilis* Ehrenberg Cohn. were tested using an agar dual culture antagonism test. *T. harzianum* and *B. subtilis* were cultured onto V8 and potato dextrose agar agar plates. From these plates (antagonists), and that of the isolated *Bipolaris* plates (pathogen), blocks measuring 10mm x 5mm were cut. The blocks were placed at opposite ends on potato dextrose agar plates. This was replicated three times. After seven days the plates were observed for the appearance of inhibition bands.

The compatibility of *T. harzianum* and *B. subtilis* was determined in the same manner as described above. Compatibility measured as either a positive or negative association.

4.2.2.3 Koch's postulate

Pennisetum clandestinum runners were planted into sterilized pine bark media, with nitrogen (N): phosphorus (P): potassium (K) amendments, in 30cm-diameter pots for one month to become acclimatized. Development of potential disease symptoms or nutritional disorders were noted during this time.

Isolated *Bipolaris* sp. cultures were recultured onto potato dextrose agar and left for 10 days under a controlled environment of 20-25°C with a 12 hr UV light and 12 hr darkness to produce conidia. This was to act as the disease inoculum.

Koch's postulate is determined by the development of disease symptoms after the host plant has been inoculated with isolated disease inoculum (Zhang and Yuen, 1999). Grass plants in the pots were inoculated with a conidial suspension of *Bipolaris* sp. Inoculated plants were transferred to a dew chamber maintained at 25°C and 100% relative humidity for 48 hrs. Pots were then transferred to a greenhouse (20-25°C) with a fixed irrigation schedule. If no lesions appeared Koch's postulate would not be proved.

Lesion numbers were recorded at 4 (including 48hrs within the dew chamber), 8, 12, 16 and 20 days post inoculation, using a random 10 leaf sample per pot. The number of lesions were recorded and area under the disease progress curve (AUDPC) was determined. Final percentage disease (FD%) was also determined.

4.2.3 FIELD TRIALS

Prepared formulations of antagonistic *Bacillus* and *Trichoderma* strains were obtained for *in vivo* testing. For the 2000 trial, *Bacillus* strains were applied as a prepared liquid formulation marketed as BIOSTART®2000 (designated as BIOSTART), comprising 4 parts *B. laterosporus* Laubach; 4 parts *B. chitinosporus* LA6A and 2 parts *B. licheniformis* Weigmann. *Trichoderma* was applied as ROOTSHIELD® (designated as ROOTSHIELD), comprising *T. harzianum*. Both BIOSTART and ROOTSHIELD were obtained from Microbial Solutions[1].

BIOSTART and ROOTSHIELD were not available for the 2002 trial. Instead, *Bacillus subtilis* B69 in talcum powder (designated *Bacillus* B69) and *Trichoderma harzianum* kd in shredded wheat (designated *Trichoderma* kd), were used. *Trichoderma* kd and *Bacillus* B69 were obtained from Plant Health Products cc[2]. The product based on *Bacillus* B69 is still undergoing performance tests before commercial release.

1 Microbial Solutions (Pty) Ltd., P.O. Box 103, Kya Sand, 2163, South Africa. Tel: (+27) 11 462-2408

2 Plant Health Products cc., P.O. Box 207, Nottingham Road, 3280, South Africa. Tel: (+27) 33 263 6130

The antagonistic potential of these two microorganisms were measured against the fungicide PUNCH XTRA® (designated PUNCH XTRA), registered for various leaf spot diseases on a number of hosts (Nel *et al.*, 1999). A water control (zero microbial treatment) was also included for comparison.

Within the treatment types, dosage rates were also assessed for the level disease control. Treatments were applied at zero (i.e. control), half and the full manufacturer's recommended dose.

Field trials were conducted at Cedara(Department of Agriculture and Environmental Affairs) in the KwaZulu-Natal Midlands, 32km inland from Pietermaritzburg, South Africa.

4.2.3.1 2000 trial

Trial site

A pure stand of *P. clandestinum* with heavy infestations of *Bipolaris* was chosen as the trial area.

Trial design

The trial design was that of a randomised complete block design, with nine treatments and five replicates, laid out into 45, 1m^2 plots. A border of 0.5m was left between the plots. Randomization of this trial design was to limit variation (CV%) in results.

Trial site preparation

Prior to the trial, the area received high traffic as the camp provided a resting place for sheep requiring inoculations. The stand was cut to 50mm in height and left for disease to become re-established (approximately three weeks). The experimental area was isolated from any animal traffic for the duration of the trial period.

Treatments

Treatments included full, half and zero (control) application dosage rates for BIOSTART, ROOTSHIELD and PUNCH XTRA. Application rates of each treatment are tabulated below in Table 4.1. The zero applications, although not necessary in the trial for each

individual treatment, were included as further variables for comparison in terms of the efficiency of the treatments based on the individual replicate blocks. ROOTSHIELD was applied using 5ℓ watering cans (one per treatment), with a pouring nozzle head of 6.2cm X 7.8cm, with spray holes 1mm apart. PUNCH XTRA and BIOSTART were applied using a pressurized spray bottle with a fine spray nozzle. These treatments were mixed with a water source obtained from the infield irrigation system.

Table 4.1. Application rates for the different treatments used in the 2000 trial to determine the efficiency of biological control agents for the control of *Bipolaris* sp.

Treatment number	Treatment	Application rate administered	Manufacturer's application rate
1	Water/ zero ROOTSHIELD	0g/4ℓ	300g/500ℓ water/ 50m^2
2	Half recommended ROOTSHIELD	1.25g/4ℓ	
3	Full recommended ROOTSHIELD	2.5g/4ℓ	
4	Water/ zero PUNCH XTRA	0mℓ/4ℓ	750mℓ ha^{-1}
5	Half recommended PUNCH XTRA	0.04mℓ/4ℓ	
6	Full recommended PUNCH XTRA	0.08mℓ/4ℓ	
7	Water/ zero BIOSTART	0mℓ/4ℓ	500mℓha^{-1}
8	Half recommended BIOSTART	0.02mℓ/4ℓ	
9	Full recommended BIOSTART	0.05mℓ/4ℓ	

Observations and analysis

The trial commenced on the 1st March 2000 with an initial rating before the first application of treatments. Five ratings and re-application of treatments followed at approximately 10 day intervals. Ratings were made according to Hall's (1991) rating scale of percentage leaf area infected by *Bipolaris* sp. on *P. clandestinum* (Figure 4.1).

Ratings of a random sample of 10 leaf blades were made from each treatment plot. The sample area was also confined towards the plot centers, 40cm from the plot edges. Ratings were limited as far as possible to new growth.

Dry matter (DM) % based on the wet and dry biomass of each plot was assessed. Wet biomass (g) samples were collected using a cutting disc measuring 30cm in diameter (area = 706.9cm^2 of the 1m^2 plots). Samples again were limited to the center of the plots to eliminate the edge effect. Dry biomass (g) was determined by drying the wet samples for 48hrs at 60°C.

The AUDPC was calculated, using Genstat 5 (Anon, 2000), for each treatment type dosage. Final percentage disease was also calculated as final percentage of disease (X_1) over the initial disease (X_o) for each experimental plot. Statistical analysis of the AUDPC values, FD%, mean final wet and dry biomass and dry matter percentage were conducted using analysis of variance (ANOVA) in Genstat 5 (Anon, 2000). Due to the unbalanced trial design, ANOVA was determined using restricted maximum likelihood (REML analysis) to eliminate the treatment error between the various control treatments.

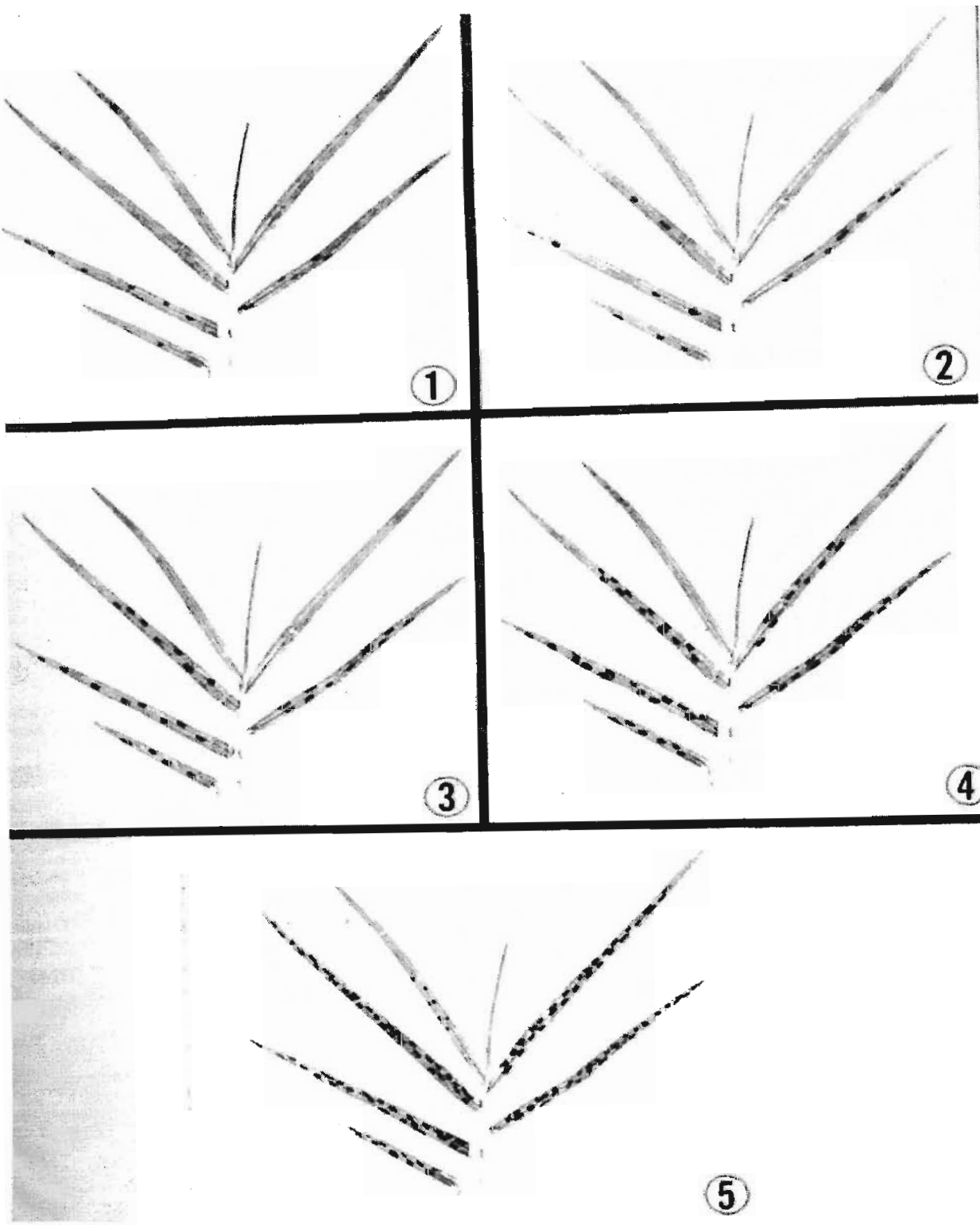


Figure 4.1 **Disease severity rating scale for Helminthosporium leaf spot (*Drechslera bicolour*) , expressed as percentage leaf area infected on the first five leaves of *Pennisetum clandestinum*; where 1= 2%, 2=4%, 3=8%, 4=16% and 5=32% (Hall, 1991).**

4.2.3.2 2002 trial

Trial site

The trial site was moved due to the low disease levels following the 2000 trial. The area comprised a pure stand of *P. clandestinum* with heavy infestations of *Bipolaris*.

Trial design

The trial design was that of a randomised complete block design, with nine treatments and four replicates, laid out into 36, 1m^2 plots. A border of 0.5m was left between the plots. The trial design was a randomised complete block design. Replications were reduced from the 2000 trial, due to the new area being smaller. A 0.5m border was allowed. Randomization of this trial design was to limit variation (CV%) in results

Trial site preparation

Prior to the trial, the area received high traffic as it served as a walkway between camps. The stand was cut to 50mm in height and left for disease to become re-established (approximately three weeks). The experimental area was isolated from any animal traffic for the duration of the trial.

Treatments

The treatments included full, half and zero (control) application rates for *Bacillus* B69, *Trichoderma* kd and PUNCH XTRA. Application rates of each treatment are tabulated below in Table 4.2. Zero application rates were included for comparison, but were tallied as a single control for all treatments.

Trichoderma kd and *Bacillus* B69 were applied using 5ℓ watering cans (one per treatment), with a pouring nozzle head of 6.2cm X 7.8cm with spray holes 1mm apart. PUNCH XTRA was applied using a pressurized spray bottle with a fine spray nozzle. These treatments were mixed with a water source obtained from the infield irrigation system.

Table 4.2. Application rates for the different treatments used in the 2002 trial to determine the efficiency of biological control agents for the control of *Bipolaris* sp.

Treatment number	Treatment	Application rate administered	Manufacturer's application rate
1	Water/ zero *Trichoderma* kd	0g/4ℓ	1g/1ℓ
2	Half recommended *Trichoderma* kd	2g/4ℓ	
3	Full recommended *Trichoderma* kd	4g/4ℓ	
4	Water/ zero PUNCH XTRA	0mℓ4ℓ	750mℓha^{-1}
5	Half recommended PUNCH XTRA	0.04mℓ/4ℓ	
6	Full recommended PUNCH XTRA	0.08mℓ/4ℓ	
7	Water/ zero *Bacillus* B69	0g/4ℓ	1g/1ℓ
8	Half recommended *Bacillus* B69	2g/4ℓ	
9	Full recommended *Bacillus* B69	4g/4ℓ	

Observations and analysis

The trial commenced on the 19th March 2002 with an initial rating before the first application of treatments. Five ratings and re-application of treatments followed at approximately 10 day intervals. Ratings were made according to Hall's (1991) rating scale of percentage leaf area infected by *Bipolaris* sp. on *P. clandestinum* (Figure 4.1).

Ratings of a random sample of 10 leaf blades were made from each treatment plot. The sample area was also confined towards the plot centers, 40 cm from the plot edges. Ratings were limited as far as possible to new growth.

Dry matter percentage based on the wet and dry biomass of each plot was assessed. Wet biomass (g) samples were collected using a cutting disc measuring 30cm in diameter (area = πr^2 = 706.9cm^2 of the 1m^2 plots). Samples again were limited to the center of the plots to eliminate the edge effect. Dry biomass (g) was determined by drying the wet samples for 48hrs at 60°C.

The AUDPC was calculated, using Genstat 5 (Anon, 2000), for each treatment type dosage. Statistical analysis of the AUDPC values, FD%, mean final wet and dry biomass and dry matter percentage was conducted using analysis of variance (ANOVA) in Genstat 5 (Anon, 2000).

4.3 RESULTS

4.3.1 SCANNING ELECTRON MICROSCOPY

Unbranched conidiophores arising from infected plant material and fungal hypha growing over the plant surface prior to penetration were observed. Hyphae appeared to outline the shape of cells, growing within the depressions at the junction of adjacent cell walls. Fungal mycelia also grew within the plant tissues, with conidiophores emerging through stomata (Plate 1, Figures 1-3).

4.3.2 *IN VITRO* TESTING

4.3.2.1 *Bipolaris* sp. isolation

From the reddish-brown elongated lesions on *P. clandestinum*, black fungal growth on potato dextrose agar was isolated (Plate 2). Submission to UV light at 12 hr intervals did not render visible polar germ tubes. Fungal growth was transferred to water agar which is nutrient poor to stimulate germ tube development (Yeun pers.com., 2002). Polar germ tubes from conidia were visible approximately 48-72 hrs later. It was also found that germ tubes developed from conidia, on both the potato dextrose agar and water agar plates, approximately 12-24 hrs after being submitted to cold temperatures (4°C). Fusiform-ellipsoidal, slightly curved, brown conidia characteristic of *Bipolaris* sp. were observed (Plate 3). The size of conidia ranged from 16-20 x 60-85µm, which is within the parameters stated by Smiley *et al.*, 1992). Protruding hilums and the bipolar germ tubes were observed.

Cladosporium was also isolated from a number of the agar plates.

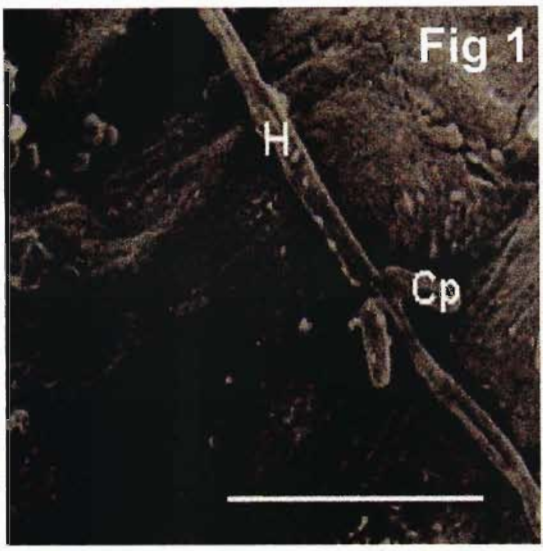


Fig 1
H
Cp

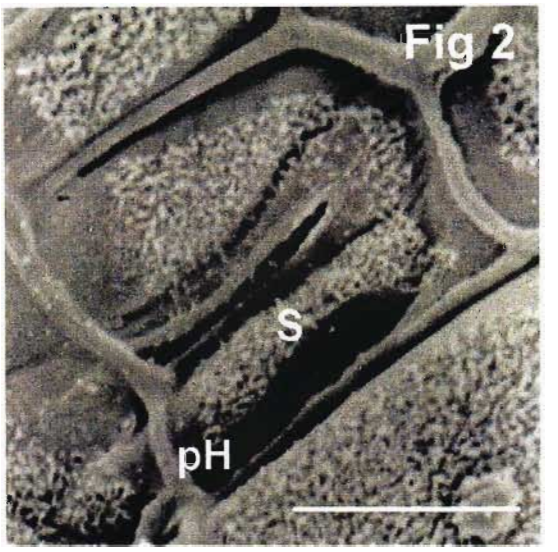


Fig 2
S
pH

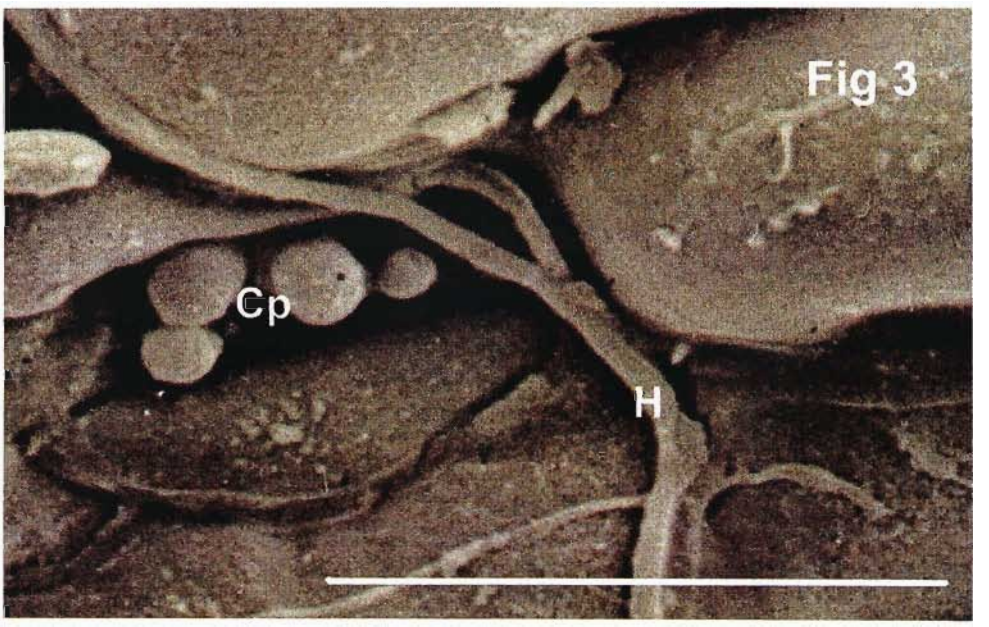


Fig 3
Cp
H

PLATE 1

Figure 1 SEM of kikuyu leaf surface showing unbranched conidiophores (Cp) from a hyphal (H) strand. (Bar = 50µm)

Figure 2 SEM of kikuyu leaf surface (unsterilized) showing the pre-penetrative hyphal (pH) growth of the fungus on the leaf surface, outlining the epidermal cells and stoma (S). (Bar = 9µm)

Figure 3 SEM of kikuyu leaf surface showing conidiophores (Cp) emerging through a stomata. (Bar = 23µm)

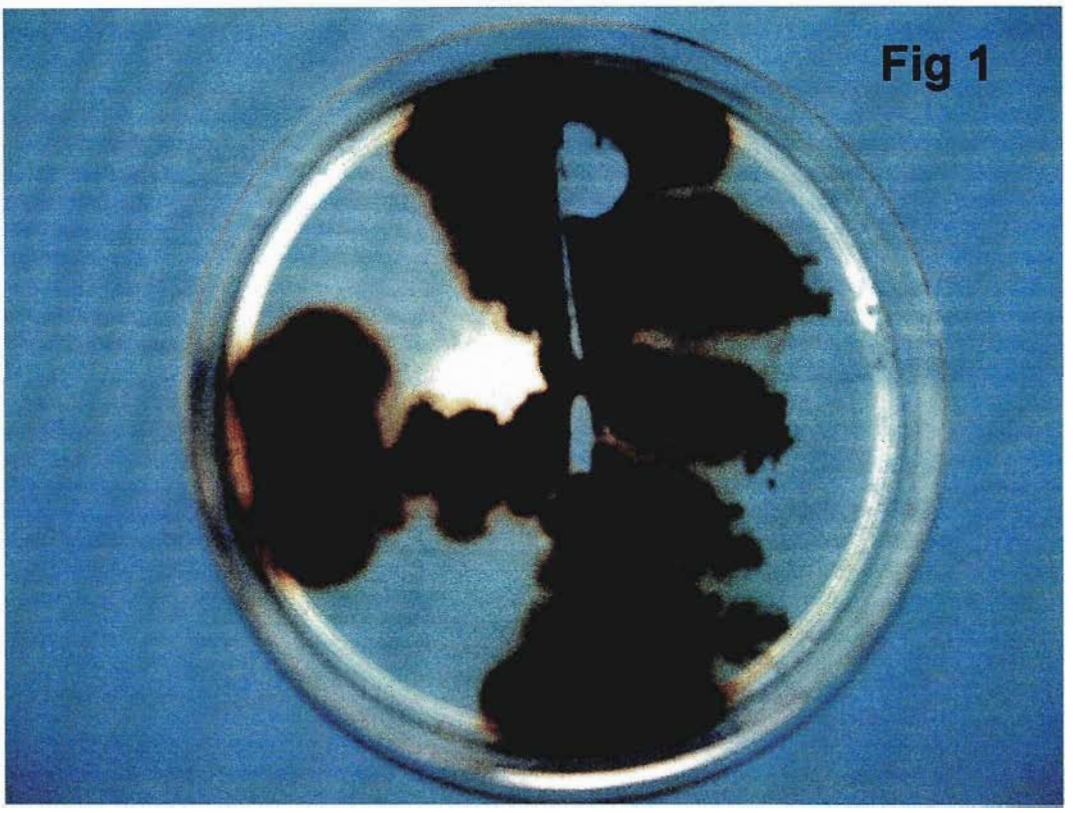


Fig 1

PLATE 2

Figure 1 **Black fungal growth, characteristic of *Bipolaris* sp., as cultured on potato dextrose agar plates and exposed to UV light at 12hr intervals to initiate sporulation.**

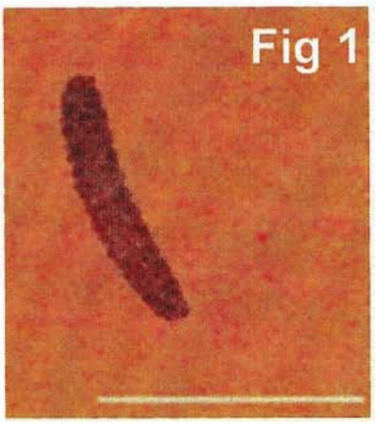


Fig 1

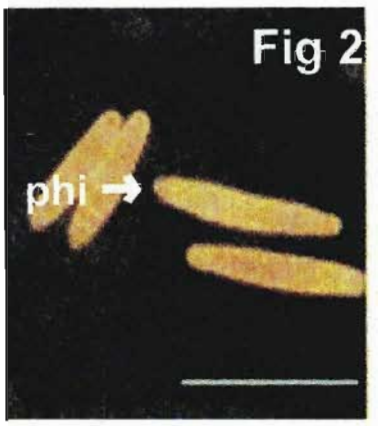


Fig 2
phi

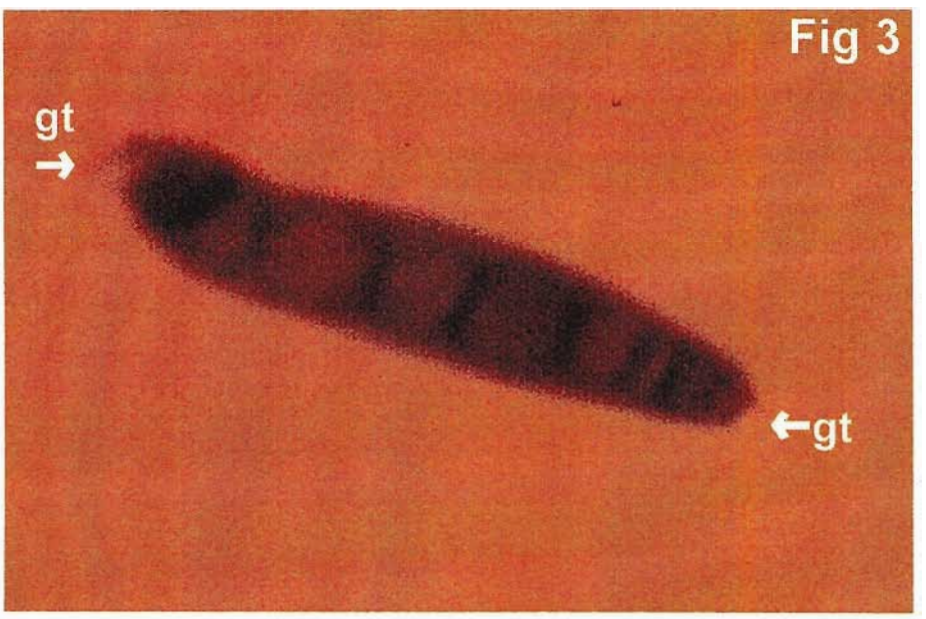


Fig 3
gt
gt

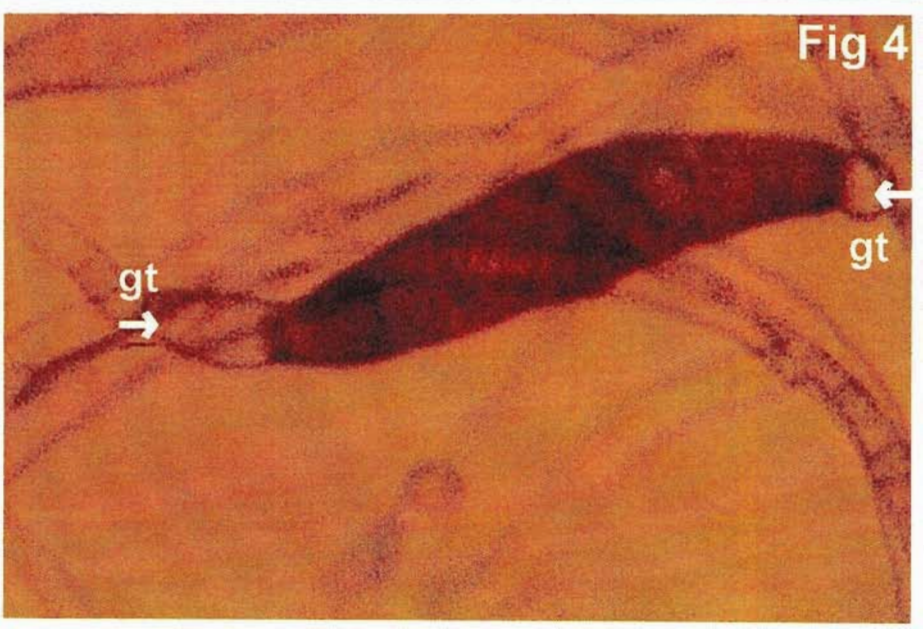


Fig 4
gt
gt

PLATE 3

Figure 1 — **Fusiform-ellipsoidal, slightly curved, brown conidium of *Bipolaris* sp. (Bar = 68µm)**

Figure 2 — **Conidia of *Bipolaris* sp. showing the protruding hilum (phi). (Bar = 68µm)**

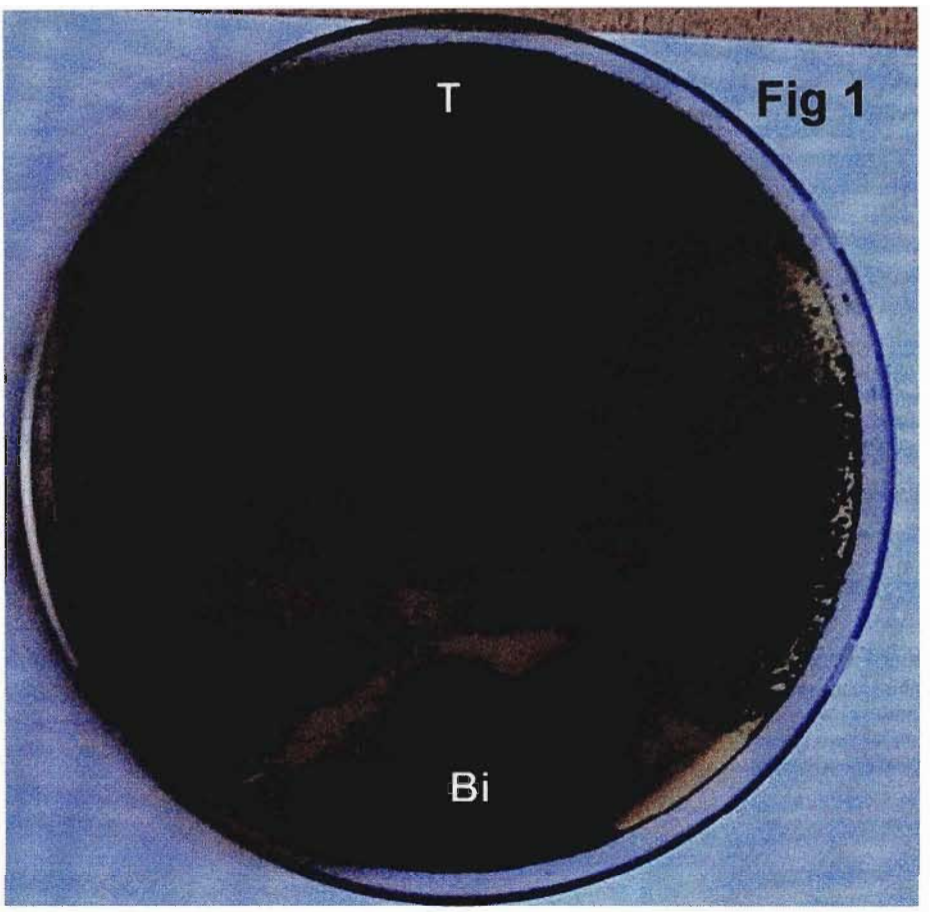
Figure 3 & 4 — **A germinating conidium of *Bipolaris* sp. showing bipolar germ tubes (gt) protruding from both end cells. (Mag x40)**

4.3.2.2 Antagonism tests

Trichoderma harzianum was seen to occupy over ⅔ of the agar plates. Distinct inhibition bands between *T.harzianum* and *Bipolaris* sp. were also clearly visible (Plate 4, Figure 1). These measured between 7-10mm in diameter.

Bacillus subtilis was seen to grow into the *Bipolaris* culture. Hyperparasitism was suspected as *Bipolaris* showed patchy growth, this being attributed to *B. subtilis* activity (Plate 4, Figure 2).

Trichoderma harzianum and *B. subtilis* were seen to be compatible in that the cultures over grew each other. However, *Trichoderma* was more vigorous than *Bacillus* in terms of growth rates as it occupied more than two thirds of the plate.


T
Fig 1
Bi

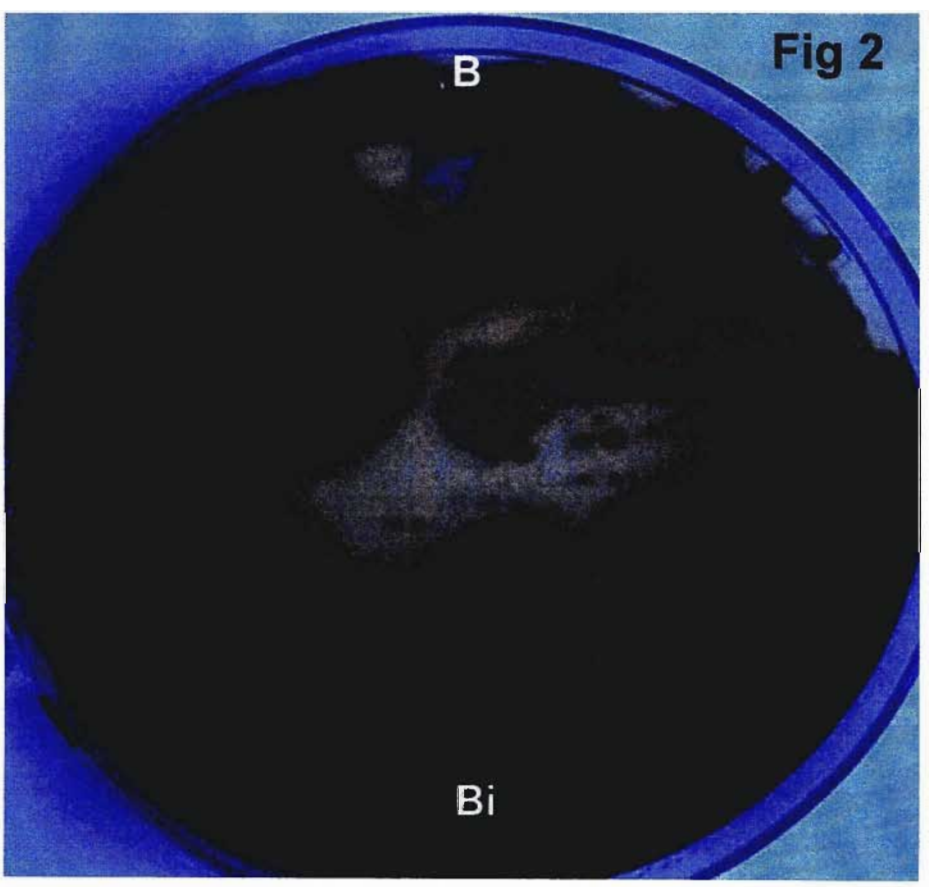

B
Fig 2
Bi

PLATE 4

Figure 1 ***Trichoderma harzianum*** **(T) (antagonist) against cultured** ***Bipolaris*** **sp. (Bi).** ***Trichoderma*** **sp. is seen to occupy over ⅔ of the plate. An inhibition band is clearly visible.**

Figure 2 ***Bacillus subtilis*** **(B) (antagonist) against cultured** ***Bipolaris*** **sp. (Bi).** ***Bacillus*** **sp. is seen to occupy only half to ⅓ of the plate. Hyperparasitism of** ***Bipolaris*** **sp. by** ***B. subtilis*** **is suspected as the two cultures grow into each other with** ***Bipolaris*** **sp. showing patchy growth.**

4.3.2.3 Koch's postulate

At four days after inoculation, including 48hrs within the dew chamber, characteristic Helminthosporium leaf spot lesions were apparent on kikuyu leaves. Plate 5 shows the characteristic lesions at 12 days post inoculation.

Disease progress is summarised in Figure 4.2. Disease development was on the uprise as plant growth increased. Occurred on new growth rather than on the older leaves and thatch present.

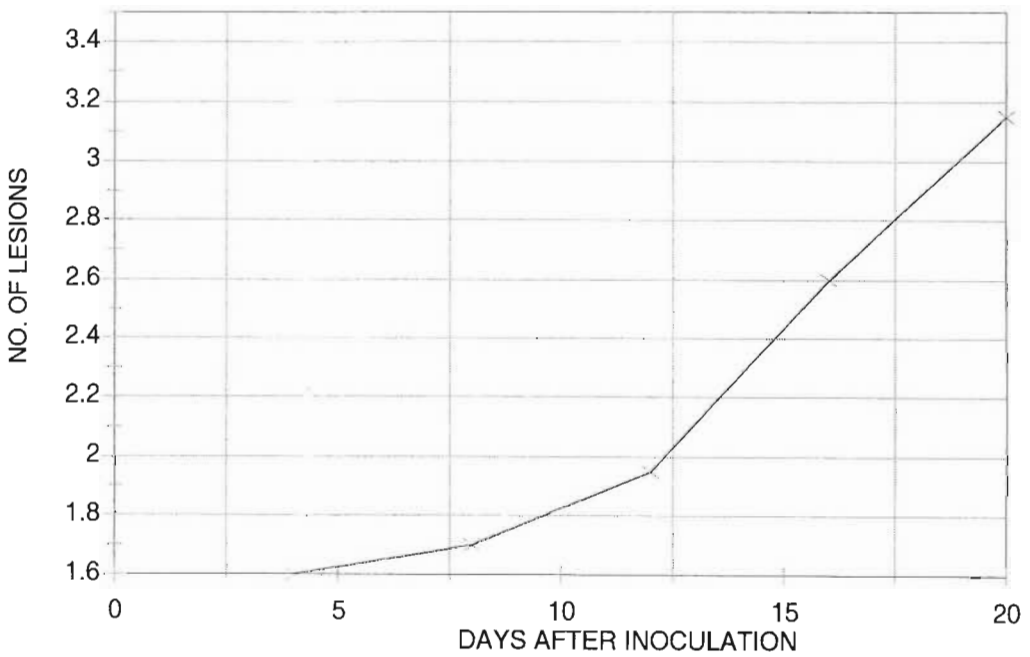


Figure 4.2 Disease progress shown as the number of Helminthosporium leaf spot lesions recorded at four day intervals until 20 days post inoculation.

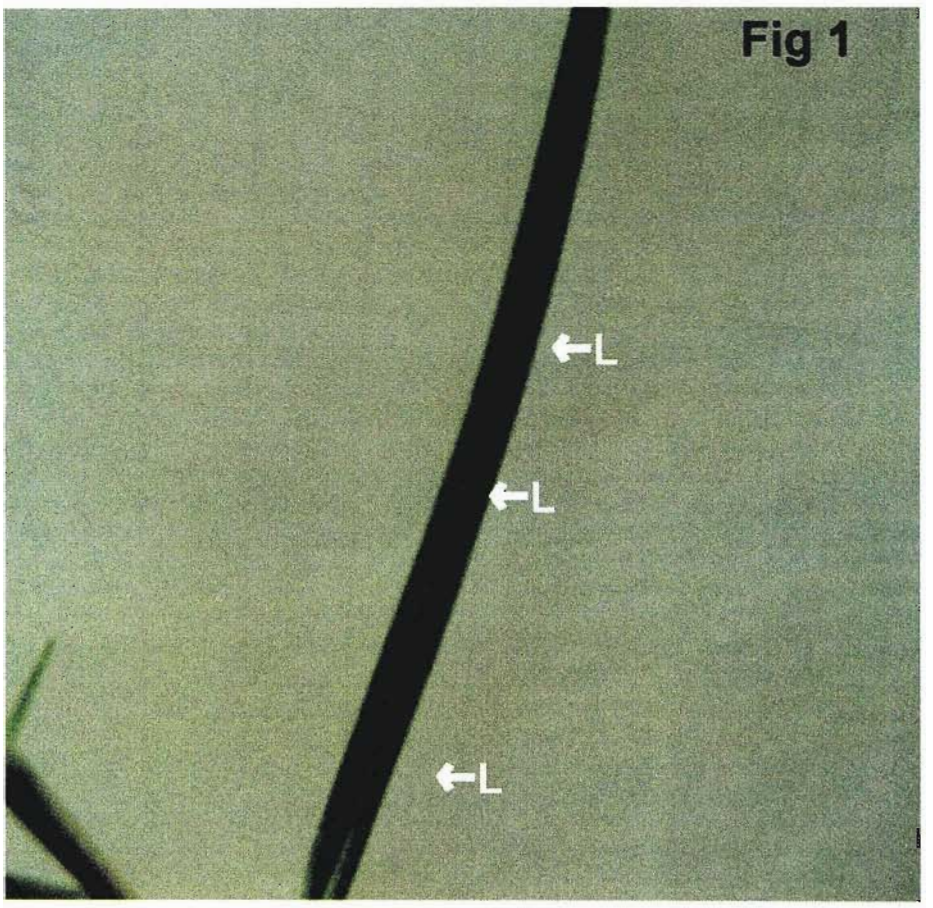


Fig 1
L
L
L

PLATE 5

Figure 1 **Characteristic reddish-brown/black lesions (L) on *Pennisetum clandestinum* (kikuyu) as observed for Koch's postulate 12 days after inoculation.**

4.3.3 FIELD TRIALS

4.3.3.1 2000 trial

The analysis of variance for AUDPC and FD% for the 2000 trial is shown in Table 4.3.

Table 4.3 Mean values of Helminthosporium leaf spot, measured as area under the disease progress curve (AUDPC) and final percentage disease for (FD%), for the 2000 trial conducted at Cedara

Treatment type doses		AUDPC	Rank within treatments	FD%	Rank within treatments
PUNCH XTRA	Zero dose	328.9	3	50.3	3
	Half dose	303.9	2	37.6	2
	Full dose	291.7	1	28.3	1
BIOSTART	Zero dose	361.7	3	57.6	3
	Half dose	297.6	1	54.8	2
	Full dose	329.1	2	46.5	1
ROOTSHIELD	Zero dose	348.4	3	48.5	3
	Half dose	304.2	2	34.2	1
	Full dose	291.8	1	38.7	2
LSD : treatment type dose			71.89		17.39

LSD at the 5% confidence limit

The application of the treatments at either a half or full dosage rate decreased disease levels in comparison to the control (as per rank within treatments, Table 4.3) over both parameters. This difference is graphically represented by Figures 4.3 and 4.4, where the treatment types, control treatments for AUDPC and FD% were greater than that of the half and full treatment doses for each treatment type.

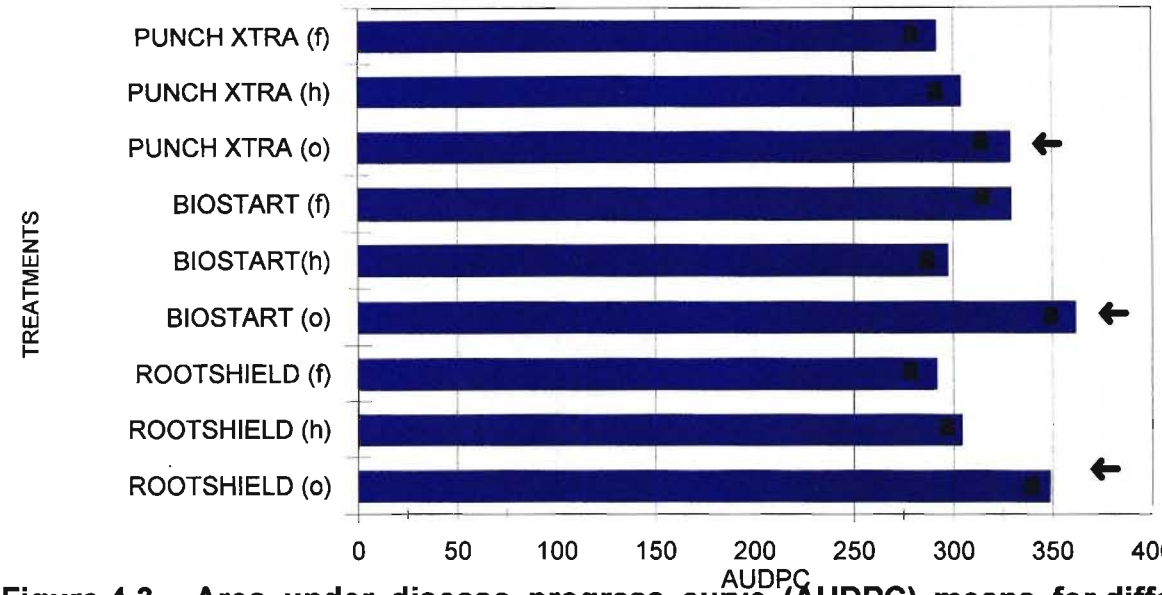


Figure 4.3 Area under disease progress curve (AUDPC) means for different treatment types at differing treatment rates used in the 2000 trial conducted Cedara (Control treatments marked with a ←).

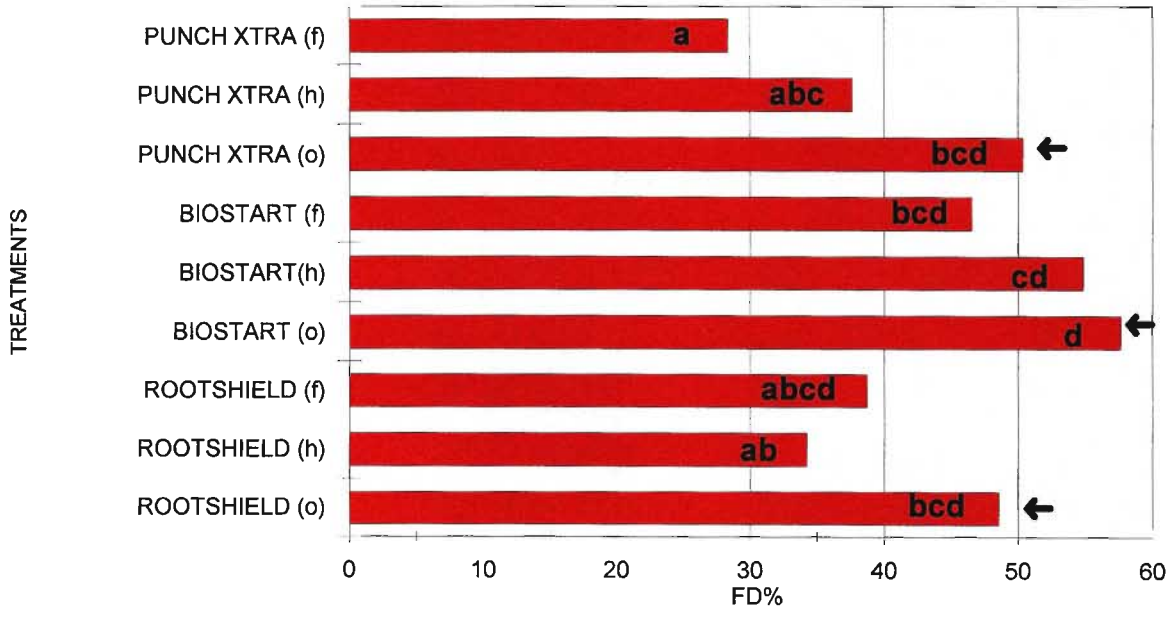


Figure 4.4 Final percentage disease (FD%) means for different treatment types at differing dosage rates used in the 2000 trial conducted at Cedara (control treatments marked with a ←).

Note: (f) = full treatment dosage rate; (h) = half and (o) = zero
AUDPC and FD% of treatments with similar letters are not significantly different from each other based on an LSD test at the 5% confidence level

Analysis of variance revealed that differences between a mean of all control and dosage treatments across all treatments, was significant ($P \leq 0.05$) (Table 4.4). Caused the individual treatment type dosage means, at a least significant difference ($LSD_{(0.05)}$) of 71.89, there was a no significant difference between the dosage rates of the treatments (Table 4.3).

In terms of FD%, a significant difference ($P \leq 0.05$) between a mean of all dosages across all treatments, as well as between the treatment types existed (Table 4.4). From Table 4.3, at a $LSD_{(0.05)} = 17.39$, a significant difference ($P \leq 0.05$) was shown between the zero dosage treatment of PUNCH XTRA and the full dosage rate only.

Table 4.4 ANOVA of area under the disease progress curve (AUDPC) and final percentage disease (FD%), for the 2000 trial conducted at Cedara

Stratum	df	Mean square		F value		F Probability	
		AUDPC	FD%	AUDPC	FD%	AUDPC	FD%
Whole plot	2	17807	9086	0.43	4.55	0.664 (NS)	0.048*
Residual	8	41239	1997	1.63	1.16		
Sub plot	2	93942	8009	3.71	4.66	0.039	0.020*
• **linear**	1	133268	15257	5.26	8.87	0.031	0.007**
• **quadratic**	1	54616	761	2.16	0.44	0.155 (NS)	0.512 (NS)
Whole plot.sub plot	4	9859	1191	0.39	0.69	0.814 (NS)	0.605 (NS)
• **linear**	2	4067	1121	0.16	0.65	0.853 (NS)	0.530 (NS)
• **quadratic**	2	15650	1261	0.62	0.73	0.547 (NS)	0.491 (NS)
Replicates	4	164655	1599	3.99	0.80		
Residual	24	25323	1720	2.31	1.20		
CV%		15.9	29.8				

REML analysis of the calculated means represents a single value for the controls of all treatment types (Table 4.5). Significant differences were noted in terms of FD%, where at an $LSD_{(0.05)}$ = 17.86 PUNCH XTRA and the control differed significantly and at $LSD_{(0.05)}$= 15.36 BIOSTART and PUNCH XTRA differed significantly. An apparent trend of increased disease control associated with the treatment types for AUDPC and FD%, was obvious.

In terms of AUDPC, the differences within the treatment types and between the individual treatment types and the control were non-significant based on the LSD values.

Table 4.5 Mean values of Helminthosporium leaf spot, measured as the area under the disease progress curve (AUDPC) and final percentage disease(FD%), for the 2000 trial conducted at Cedara , based on REML statistical analysis

			Treatment types			Control
			ROOTSHIELD	BIOSTART	PUNCH XTRA	
AUDPC	Treatment type. dose means	Half dose	303.9	295.1	306.7	-
		Full dose	291.4	326.6	294.6	-
	LSD : treatment type dose		54.94			64.81
	Treatment type means		297.7	310.9	300.7	346.3
	Rank of treatment types		1	3	2	4
	LSD : treatment type		60.33			71.16
FD%	Treatment type. dose means	Half dose	34.28	54.73	37.62	-
		Full dose	38.77	46.41	28.33	-
	LSD : treatment type dose		13.98			16.27
	Treatment type means		36.53	50.57	32.98	52.10
	Rank of treatments types		2	3	1	4
	LSD : treatment type		15.36			17.86

LSD at the 5% confidence limit

Analysis of variance indicated that all of the treated plots differed significantly ($P \leq 0.05$) from the untreated control plots, for both AUDPC and FD% (Table 4.6). Analysis also reiterated the significant difference ($P \leq 0.05$) within the treatment types and between the control and treatment types of FD%. Based on LSD values presented in Table 4.5 for the treatment type doses for FD%, the control treatment differed significantly from the half dosage rate of ROOTSHIELD and from the full dosage rate of PUNCH XTRA.

The dosage rates, for both AUDPC and FD%, were non-significant ($P \geq 0.05$) as per REML analysis.

Table 4.6 REML table of area under the disease progress curve (AUDPC) and final percentage disease(FD%) for the 2000 trial conducted at Cedara

Stratum	df	Wald statistic		F Probability	
		AUDPC	FD%	AUDPC	FD%
Treatment	1	7.59	8.64	0.006 **	0.003 **
• **Types**	2	0.29	9.10	0.863 (NS)	0.011 **
• **Doses**	1	0.02	0.85	0.899 (NS)	0.357 (NS)
• **Type.dose**	2	1.29	1.75	0.524 (NS)	0.416 (NS)
CV%		6.38	4.73		

Analysis of variance, using a single mean control for all the treatment types used for REML analysis, revealed a significant difference ($P \leq 0.05$) between the treatment types and control, and a significant difference ($P \leq 0.05$) within the treatment types caused dosage rates (Table 4.7).

The co-efficient of variance (CV%) was very high rendering significance unlikely.

Table 4.7 ANOVA of final percentage disease (FD%) for the 2000 trial conducted at Cedara, to determine the relationship between treatment types and dosage rates Note: a mean control over all treatments was used in this analysis

Stratum		df	Mean Square	F value	F probability
Treatments		6	6103	4.52	0.003**
ROOTSHIELD	• **linear**	1	12238	9.06	0.006**
	• **quadratic**	1	4759	3.52	0.073 (NS)
Deviations		4	4905	3.63	0.019 (NS)
BIOSTART	• **linear**	1	6614	4.9	0.037*
	• **quadratic**	1	144	0.11	0.747 (NS)
Deviations		4	7465	5.53	0.003**
PUNCH XTRA	• **linear**	1	22396	16.58	< 0.001***
	• **quadratic**	1	109	0.08	0.779 (NS)
Deviations		4	3528	2.61	0.061 (NS)
Replicates		4	1599	1.18	
Residual		34	1351	0.92	
CV%			86.8		

Linear fit disease curves are shown in Figures 4.5, 4.6 and 4.7 for each treatment type and dosage rate within the treatment. Regression analysis confirmed that data (ratings) fitted a negative linear curve.

However, based on the ANOVA test in Table 4.4, the curve gradients (treatment type dose) were not statistically different from each other. The regression lines did, however, show a trend of improved disease control with the application of treatments at either a full or half dosage rate, in comparison to the zero dosage rate (control).

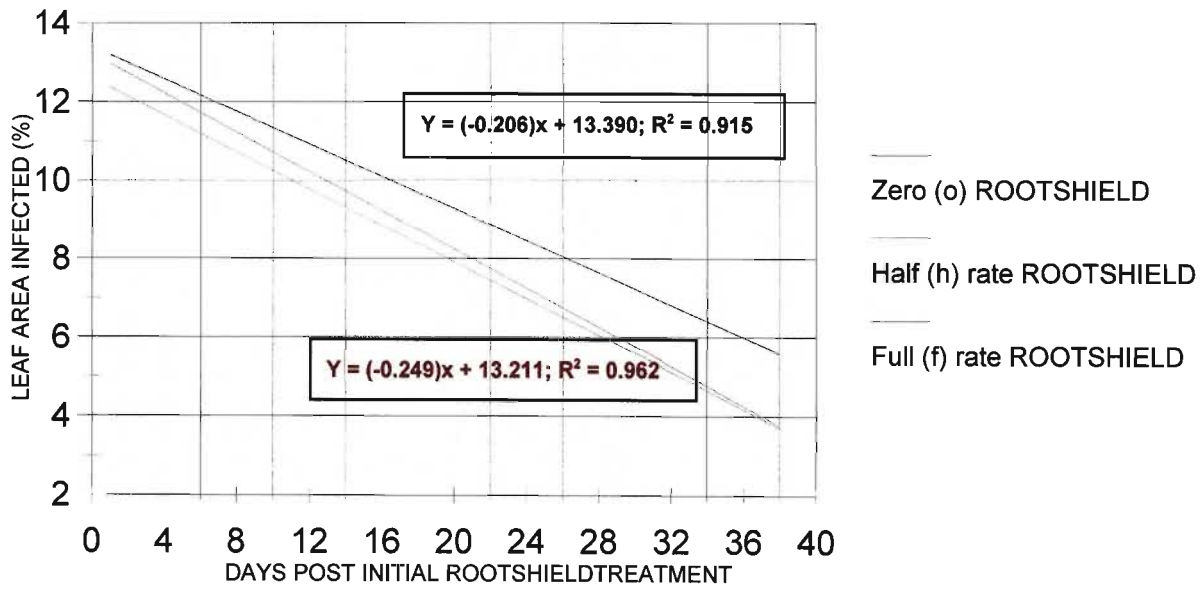


Figure 4.5 Linear fit of the disease progress curve for the different dosage rates of ROOTSHIELD (*T. harzianum*) used in the 2000 trial conducted at Cedara.

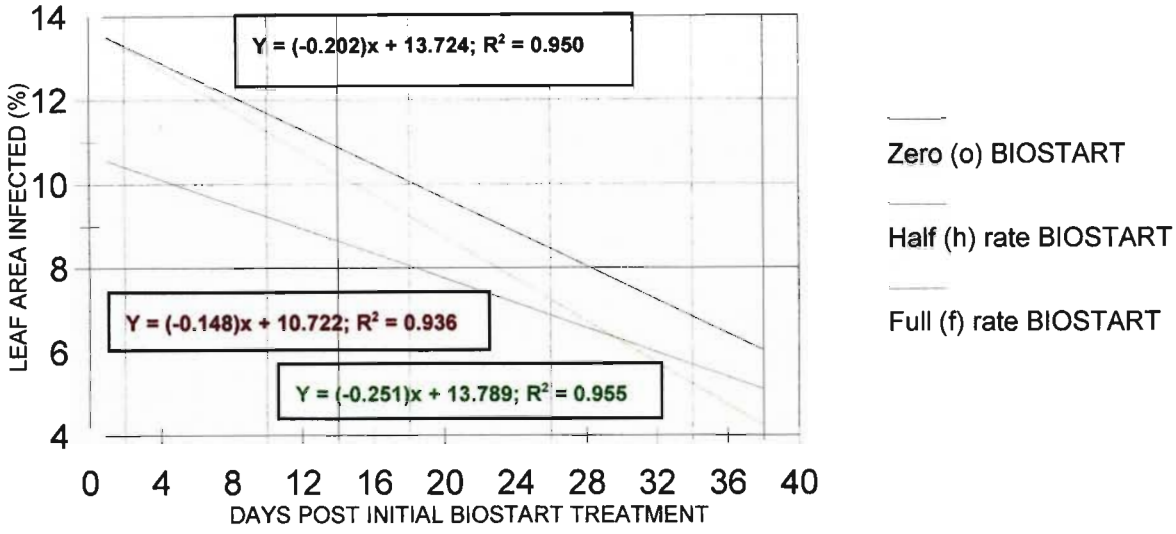


Figure 4.6 **Linear fit of the disease progress curve for the different dosage rates of BIOSTART (*Bacillus* sp.) used in the 2000 trial conducted at Cedara.**

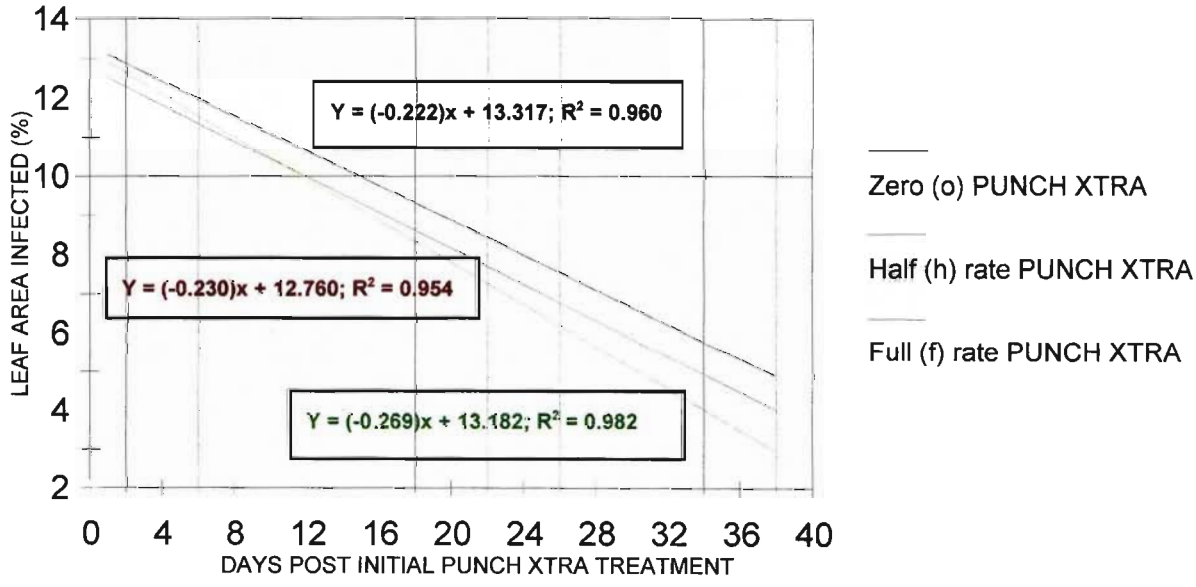


Figure 4.7 **Linear fit of the disease progress curve for the different dosage rates of PUNCH XTRA (fungicide) used in the 2000 trial conducted at Cedara.**

The effect of the occurrence of Helminthosporium leaf spot on plant growth rates, in terms of wet weight, dry weight and dry matter percentages for each treatment type dose, is summarised in Table 4.8.

Table 4.8 Mean values of Helminthosporium leaf spot, measured as wet weight (WW), dry weight (DW) and dry matter percentage (DM%) for the 2000 trial conducted at Cedara

Treatment type dose		WW	Rank within treatments	DW	Rank within treatments	DM%	Rank within treatments
PUNCH XTRA	**Zero dose**	1.81	1	0.854	3	39.13	1
	Half dose	1.93	2	0.808	1	43.93	3
	Full dose	2.05	3	0.836	2	41.50	2
BIOSTART	**Zero dose**	1.57	1	0.918	3	60.46	3
	Half dose	1.92	3	0.794	1	41.11	1
	Full dose	1.82	2	0.884	2	54.53	2
ROOTSHIELD	**Zero dose**	1.99	2	0.894	3	45.70	2
	Half dose	2.28	3	0.829	2	40.74	1
	Full dose	1.69	1	0.812	1	48.88	3
LSD: treatment type dose		0.548		0.1813		16.34	

LSD at the 5% confidence limit

From Table 4.8, the control treatments of PUNCH XTRA and BIOSTART accounted for the lowest wet weight, but the greatest dry weight and dry matter percentage. Based on $LSD_{(0.05)}$=16.34 for dry matter percentage, the difference between the BIOSTART control treatment and the half dosage rate of BIOSTART, was the only significant difference ($P \leq 0.05$). This significant difference ($P \leq 0.05$) of dosage rates was confirmed by the ANOVA test (Table 4.9).

Table 4.9 ANOVA of dry matter percentage determined from wet and dry weights (g) measured for the 2000 trial conducted at Cedara

Stratum	df	Mean Square	F value	F probability
Whole plot	2	160.2	0.79	0.488 (NS)
Residual	8	203.7	1.49	
Sub plot	2	509.1	3.73	0.039*
• Linear	1	207.1	1.52	0.230 (NS)
• Quadratic	1	811.1	5.95	0.023*
Whole plot.sub plot	4	106.9	0.78	0.547 (NS)
• Linear	2	114.0	0.84	0.446 (NS)
• Quadratic	2	99.9	0.73	0.491 (NS)
Replicates	4	276.7	1.36	
Residual	24	136.4		
CV%		24.4		

In terms of dry weight, the whole plot variance was smaller than the residual error mean square and thus the randomised block design ANOVA was valid (Table 4.10).

Analysis of variance of the treatment types.dose revealed no significant differences ($P \geq 0.05$) between the control and the treatment dosages, or between the treatment types and the control for dry weights.

Table 4.10 ANOVA of dry weights for the 2000 trial conducted at Cedara, to determine the relationship between treatment types and dosage rates

Note: a mean control over all treatments has been used in this analysis

Stratum		df	Mean Square	F value	F probability
Treatments		6	0.01260	0.63	0.703 (NS)
ROOTSHIELD	• **linear**	1	0.04511	2.27	0.141 (NS)
	• **quadratic**	1	0.00305	0.15	0.693 (NS)
Deviations		4	0.00685	0.34	0.846 (NS)
BIOSTART	• **linear**	1	0.01613	0.81	0.374 (NS)
	• **quadratic**	1	0.04482	2.25	0.143 (NS)
Deviations		4	0.00366	0.18	0.945 (NS)
PUNCH XTRA	• **linear**	1	0.03383	1.70	0.201 (NS)
	• **quadratic**	1	0.01410	0.71	0.406 (NS)
Deviations		4	0.00691	0.35	0.844 (NS)
Replicates		4	0.04596	2.31	
Residual		34	0.01990		
CV%			16.6		

The whole plot variance for dry and wet weight and dry matter percentage were removed and re-analysed using REML statistical test (Table 4.11).

Control treatments ranked lower than the treatment types for wet weight, but higher in terms of dry weight and dry matter percentage. The mean treatment types varied for each parameter (Table 4.11). Significant differences at the 5% confidence level, were noted for wet weights between the half and full dosage rate of ROOTSHIELD, based on a $LSD_{(0.05)}$ =0.443, the mean control and the half dosage rate of ROOTSHIELD at $LSD_{(0.05)}$ = 0.515. Dosage rates of BIOSTART differed significantly in terms of the dry matter percentage, at $LSD_{(0.05)}$ = 12.78.

Table 4.11 Mean values of Helminthosporium leaf spot, measured as final wet weight, dry weight and dry matter for the 2000 trial conducted at Cedara, based on REML analysis

			Treatment types			Control
			ROOTSHIELD	BIOSTART	PUNCH XTRA	
Wet weight (g)	Treatment type. dose means	Half dose	2.278	1.921	1.932	-
		Full dose	1.688	1.821	2.052	-
	LSD: treatment type dose		0.443			0.515
	Treatment type means		1.983	1.871	1.992	1.727
	Rank of treatment types		3	2	4	1
	LSD: treatment type		0.487			0.566
Dry weight (g)	Treatment type. dose means	Half dose	0.8300	0.7900	0.8100	-
		Full dose	0.8120	0.8840	0.8360	-
	LSD: treatment type dose		0.1487			0.1727
	Treatment type means		0.8210	0.8370	0.8230	0.8973
	Rank of treatment types		1	3	2	4
	LSD: treatment type		0.1487			0.1727
Dry matter %	Treatment type. dose means	Half dose	40.74	41.11	43.93	-
		Full dose	48.88	54.53	41.50	-
	LSD: treatment type dose		12.78			15.04
	Treatment type means		44.81	47.82	42.72	53.56
	Rank of treatment types		2	3	1	4
	LSD: treatment type		14.03			16.52

LSD at the 5% confidence limit

Analysis of variance showed a significant ($P \leq 0.05$) difference between the mean of the treated and untreated plots (Table 4.12).

There was no significance ($P \geq 0.05$) differences noted for the treatment type doses.

Table 4.12 REML table of wet weight (WW), dry weight (DW) and dry matter percentage (DM%) for the 2000 trial conducted at Cedara

Stratum	df	Wald statistic			F Probability		
		WW (g)	DW (g)	DM (%)	WW (g)	DW (g)	DM (%)
Treatment	1	2.85	2.29	5.28	0.091 (NS)	0.115 (NS)	0.022
• Types	2	0.51	0.17	0.77	0.775 (NS)	0.963 (NS)	0.682 (NS)
• Doses	1	1.57	0.40	2.26	0.210 (NS)	0.509 (NS)	0.133 (NS)
• Type.Dose	2	3.83	0.74	2.41	0.147 (NS)	0.670 (NS)	0.300 (NS)
CV%		6.38	4.11	5.87			

4.3.3.2 2002 trial

Due to the validity of the randomized trial design, ANOVA was the only statistical analysis used.

Means separation is summarised in Table 4.13. Between treatment types, PUNCH XTRA accounted for the lowest disease incidence for both AUDPC and FD%. Disease determined by AUDPC, was higher for treatments *Trichoderma* kd and *Bacillus* B69 in comparison to the control. This occured again for *Trichoderma* kd, in terms of FD%. Within the treatment type doses, it was only within the PUNCH XTRA treatment for both AUDPC and FD%, as well as the FD% of *Bacillus* B69, that the control accounted for the highest disease incidence. In the AUDPC of *Trichoderma* kd treatment type doses, the control accounted for lowest disease incidence.

Table 4.13 Mean values of Helminthosporium leaf spot, measured as the area under the disease progress curve (AUDPC) and final percentage disease (FD%) for the 2002 trial conducted at Cedara

			Treatment types						Control
			Trichoderma kd	R[1]	*Bacillus* B69	R[1]	PUNCH XTRA	R[1]	
AUDPC	Treatment type dose means	Zero dose	256.8	1	256.8	2	256.8	3	-
		Half dose	346.7	2	315.4	3	161.2	1	-
		Full dose	361.0	3	238.2	1	197.6	2	-
	LSD: treatment type dose		165.3						
	Treatment type means		353.9		276.8		179.4		256.8
	Rank of treatment types		4		3		1		2
	L.S.D.: treatment types		116.9						106.7
FD%	Treatment type dose means	Zero dose	55.0	2	55.0	3	55.0	3	-
		Half dose	38.1	1	48.8	1	33.2	1	-
		Full dose	80.8	3	51.9	2	40.0	2	-
	LSD: treatment type dose		51.0						
	Treatment type means		59.5		50.3		36.6		55.0
	Rank of treatment types		4		2		1		3
	L.S.D.: treatment types		51.1						44.3

LSD at the 5% confidence limit for the doses (sub plots)
R[1] Rank of doses within the treatments

These results are graphically represented in Figures 4.8 and 4.9. Results confirmed that full dosage rates of *Trichoderma* kd accounted for the lowest disease control over all of the treatments, for both AUDPC and FD%.

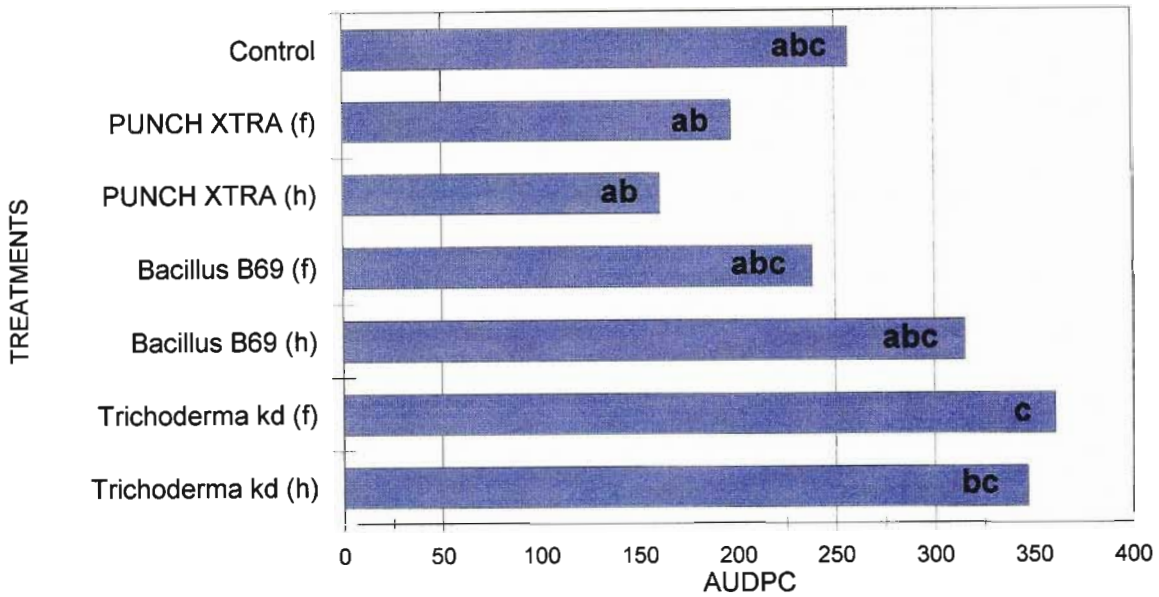


Figure 4.8 Area Under Disease Progress Curve (AUDPC) means for different treatment types at differing treatment dosage rates used in the 2002 trial conducted at Cedara.

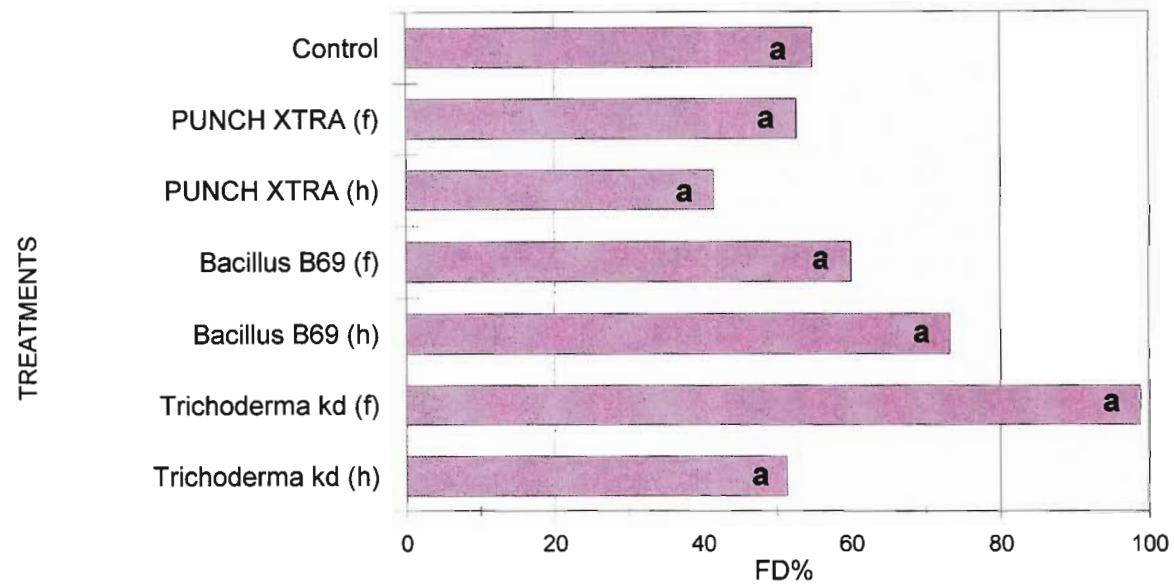


Figure 4.9 Final percentage disease (FD%) means for different treatment types at differing treatment dosage rates used in the 2002 trial conducted at Cedara.

Note: (f) = full treatment dosage rate and (h) = half
AUDPC and FD% of treatments with similar letters are not significantly different from each other based on an LSD test at the 5% confidence level

Analysis of variance showed significance ($P \leq 0.05$) differences between the AUDPC treatment types (Table 4.14). From Table 14.4, based on $LSD_{(0.05)}$ = 116.9, this significant difference existed between PUNCH XTRA and *Trichoderma* kd treatment types.

No statistical differences ($P \geq 0.05$) were noted for the dosage rates of treatment types.

Table 4.14 ANOVA of area under the disease progress curve (AUDPC) and the final percentage disease(FD%) for the 2002 trial conducted at Cedara

Note: CV% based on log data transformation

Stratum	df	Mean square		F value		F Probability	
		AUDPC	FD%	AUDPC	FD%	AUDPC	FD%
Treatments	1	0.1254	0.2893	0.06	0.68	0.817 (NS)	0.420 (NS)
• **Types**	2	14.1166	0.7147	6.24	1.68	0.009**	0.214 (NS)
• **Doses**	1	0.6551	0.2402	0.29	0.57	0.597 (NS)	0.462 (NS)
• **Type.Dose**	2	0.3447	0.5085	0.15	1.20	0.860 (NS)	0.325 (NS)
Replicate	3	13.0241	1.3157	5.75	3.10		
Residual	18	2.2638	0.4248	11.64			
CV%		8.2	17.9				

Linear fit disease curves, based on the treatment type dose rates, are shown in Figures 4.10, 4.11 and 4.12. Regression analysis confirmed that the data (ratings) fitted a negative linear curve, except for the full dosage rate of *Trichoderma* kd. The regression lines showed a trend of decreasing disease at a more rapid rate (based on the linear gradients) with the half and full dosage rates of *Bacillus* B69 and PUNCH XTRA, and the half dosage rate of *Trichoderma* kd, when compared to the zero treatment (control).

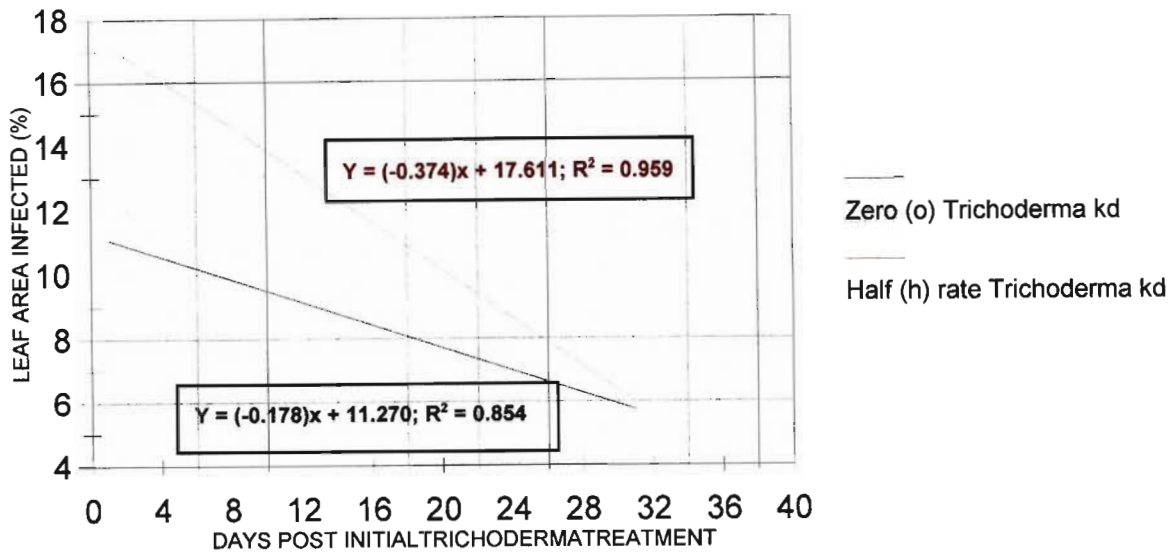


Figure 4.10 Linear fit of the disease progress curve for the different rates of *Trichoderma* kd (*T. harzianum)* used in the 2002 trial conducted at Cedara.

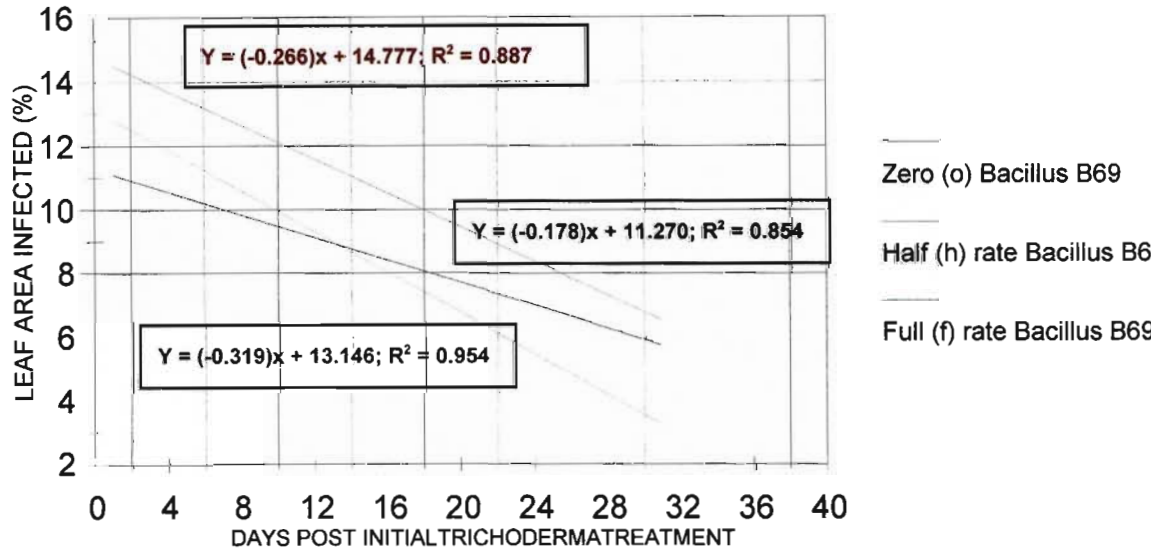


Figure 4.11 Linear fit of the disease progress curve for the different rates of *Bacillus* B69 (*B. subtilis*)used in the 2002 trial conducted at Cedara.

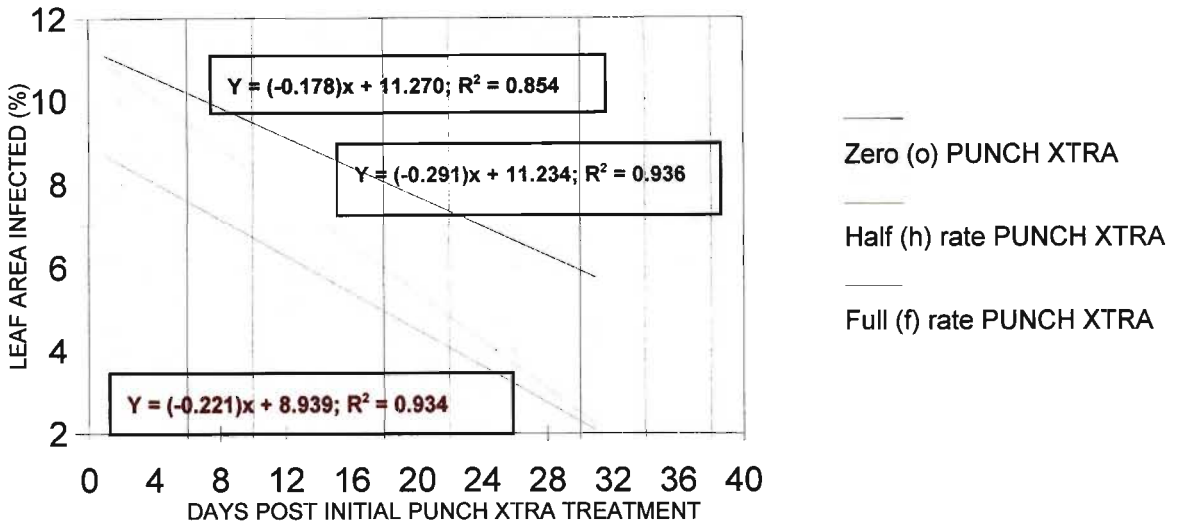


Figure 4.12 Linear fit of the disease progress curve for the different rates of PUNCH XTRA (fungicide) used in the 2002 trial conducted at Cedara.

The effect of Helminthosporium leaf spot, induced by *Bipolaris* sp., on growth rates is expressed in Table 4.15. Based on a $LSD_{(0.05)} = 0.563$ the only significant difference was between wet weight of the control and half dosage rate of PUNCH XTRA.

No specific trend was visible in terms of ranking treatment types.doses, except for wet weights where the full dosage rates of all treatment types accounted for the lowest biomass (g). This was true also for dry weight of the full dosage rate of PUNCH XTRA.

It was noted that the control never accounted for the highest disease incidence.

Table 4.15 Mean values of *Helmithsporium* leaf spot, measured as wet weight, dry weight and dry matter for the 2002 trial conducted at Cedara

			Treatment types						
			Trichoderma kd	R[1]	*Bacillus* B69	R[1]	PUNCH XTRA	R[1]	Control
Wet weight (g)	Means for doses	Zero dose	2.927	2	2.927	3	2.927	2	-
		Half dose	3.037	3	2.913	2	3.565	3	-
		Full dose	2.718	1	2.715	1	2.900	1	-
	LSD: treatment type dose		0.689						0.563
	Treatment type means		2.878		2.814		3.233		2.927
	Rank of treatment types		2		1		4		3
	L.S.D.: treatment types		0.488						0.449
Dry weight (g)	Means for doses	Zero dose	0.945	1	0.945	3	0.945	2	-
		Half dose	0.990	3	0.815	1	1.063	3	-
		Full dose	0.950	2	0.915	2	0.925	1	-
	LSD: treatment type dose		0.261						0.214
	Treatment type means		0.970		0.865		0.994		0.945
	Rank of treatment types		3		1		4		2
	L.S.D: treatment types		0.185						0.169
Dry matter %	Means for doses	Zero dose	32.45	1	32.45	2	32.45	3	-
		Half dose	32.72	2	29.36	1	30.34	1	-
		Full dose	35.69	3	33.80	3	32.02	2	-
	LSD: treatment type dose		7.75						6.33
	Treatment type means		34.20		31.58		31.18		32.45
	Rank of treatment types		4		2		1		3
	L.S.D.: treatment types		5.48						5.01

LSD at the 5% confidence limit for the doses (sub plots)

R[1] Rank of doses within the treatments

Analysis of variance of the weights and dry matter percentage showed no significance ($P \geq 0.05$) (Table 4.16).

Table 4.16 ANOVA of wet weight (WW), dry weight (DW) and dry matter (DM%) for the 2002 trial conducted at Cedara

Stratum	df	Mean Square			F value			F probability		
		WW (g)	DW (g)	DM (%)	WW (g)	DW (g)	DM (%)	WW (g)	DW (g)	DM (%)
Treatments	1	0.0184	0.00003	0.19	0.08	0.00	0.01	0.777(NS)	0.974(NS)	0.935(NS)
•Types	2	0.4073	0.03755	21.57	1.81	1.16	0.76	0.184(NS)	0.329(NS)	0.479(NS)
•Dose	1	0.9322	0.00400	55.02	4.15	0.12	1.93	0.052(NS)	0.728(NS)	0.176(NS)
•Type.Dose	2	0.1175	0.02850	3.80	0.52	0.88	0.13	0.599(NS)	0.426(NS)	0.875(NS)
Replicate	3	0.3082	0.06492	155.03	1.37	2.01	5.45			
Residual	26	0.2249	0.03235	28.45						
CV%		16.0	19.1	16.5						

4.4 DISCUSSION

Overview of Helminthosporium leaf spot on kikuyu at Cedara

Disease incidences detract from a plant's production potential. Helmithosporium leaf spot is a commonly encountered disease of both pasture and turf grasses in the KwaZulu-Natal Midlands of South Africa, especially within the Moist Midlands Mistbelt (Bioresource Group 5) (Camp, 1995). The genus *Helminthosporium* was established in 1809 (Alcorn, 1988). Graminicolous *Helminthosporium* is now referred to by species within the genera: *Bipolaris, Drechslera* and *Exserohilum*. These genera are closely related and differentiated by conidial shape, hilum and the origin of the germ tubes (Smiley *et al.,* 1992; Couch, 1995). Using these, the genera of Helminthosporium leaf spot occurring at Cedara, was confirmed as being *Bipolaris* sp., the origin of the bipolar germ tubes (Plate 3; Figure 3 and 4) confirming the identification .

Disease symptoms vary depending on the grass species invaded. In general symptoms include reddish-brown to purplish-black lesions, measuring 1-4mm on the grass leaves (Figure 4.13). These leaf spots are more concentrated near to the collar of the leaf blade (Lenné, 1994). In severe disease cases the crowns, stems and rhizomes are also infected, resulting in chlorosis and wilting of the leaves and the infected plant dies (Schroeder and Sprague, 1996). Kikuyu being the predominate pasture grass used in the Midlands, was studied as a host. At Cedara, Helminthosporium leaf spot occurs from September on kikuyu pastures. This coincides with the onset of spring rains and more humid, overcast and misty conditions. Disease prevails throughout the summer, with new outbreaks tailing off as winter approaches. Disease symptoms should be less apparent when pastures become dormant, dry and frosted, however, disease symptoms were seen to prevail throughout the winter period (June/July) at Cedara. This may simply have been the expression of the residual "spring/summer" symptoms.

Figure 4.13 A severe infestation of dark reddish-brown lesions measuring 1-4mm associated with Helminthosprium leaf spot. Chlorosis (yellowing) and die-back (browning) are associated with severe infestations (Cedara, 2002).

Although disease is more prevalent with every new growth season of kikuyu pastures in the Midlands, it is not considered worth the expense or effort to control further disease spread via means of chemical sprays. This was the standing mindset amongst many farmers (Chapter 3). Farmers are, however, being forced to consider disease control options available to them with the onset of new diseases which are developing in the Midlands, namely kikuyu yellows and ryegrass blast.

Symptoms of Helminthosporium leaf spot (Figure 4.13) are similar to a number of leaf spot diseases, with farmers often mistaking the disease for rust. Rust is easily identified by the presence of pustules of which were not observed on kikuyu at the Cedara trial sites. However, using SEM techniques and Zeiss light microscope, unbranched

conidiophores (Plate 1) bearing single fusiform conidia were observed (Plate 2). Conidia of *Bipolaris* are characteristically fusiform (Smiley *et al.*, 1992). Mycelium was also seen to grow along the plant surface (Plate 1, Figure 2). Internal hyphal growth was confirmed by conidiophores emerging through the stomata (Plate 1, Figure 3).

The occurrence of *Cladosporium* sp. is not unusual, as this fungus is an opportunistic saprophyte and colonises both dead and living plant tissues. With the onset of senescence, spores of *Cladosporium* sp. germinate (Blakeman, 1985). On grasses, *Cladosporium* sp. often accounts for seedling related diseases. However, on Timothy grass (*Phleum pratense* L.) it may cause the foliar disease, Cladosporium eyespot (Smiley *et al.,* 1992).

Potential for biological control of Helminthosporium leaf spot

Control of *Bipolaris* sp. using BCAs, *T. harzianum* and *B. subtilis*, was tested *in vitro* by means of an agar dual culture antagonism test. The use of two antagonists applied to the same area simultaneously for disease control over a broader range of environmental conditions exists (Guetsky *et al.*, 2001). The potential combination of *T. harzianum* and *B. subtilis* was confirmed in the compatibility test, as neither microbe inhibited the growth of the other. *Trichoderma harzianum* did, however, appear to be more aggressive. This may be attributed to colony growth expressed on the potato dextrose agar plates. *Bacillus subtilis* formed a smooth yellowish growth which was not easily discernable from the agar, especially when the dark-greenish fungal growth become more prominent.

Increased vigour associated with *T. harzianum*, *in vitro*, may be attributed to conditions being more conducive to fungal growth. Recorded temperatures ranged between 27-28°C. Although high temperatures are suited to the growth potential of both *Trichoderma* and *Bacillus* spp., the optimum temperature for *Bacillus* spp. is less, ranging from 20-25°C (Krebs *et al.*, 1998).

In vitro testing alone cannot confirm what is to be expected within an open system. In an open system, factors such as climatic conditions (temporal and moisture-related), natural soil suppressiveness (Weller, 1988) and the host plant (Smith and Goodman, 1999) must be considered. Field trials were therefore, conducted during two disease seasons. The trial sites were, however, different for each trial, as the first site was associated with a low disease incidence in 2001. This low disease incidence may be attributed to the previous applications of BCAs. *Trichoderma harzianum*, derived from ROOTSHIELD, is known to be persistent in the rhizosphere for long periods (Harman, 2001), increasing disease control into the second growing season (Inbar *et al.*, 1994).

In the 2000 trial, significant differences between treatments was found when FD% was used as the analysis parameter and in the 2002 trial, significance was associated with AUDPC (Table 4.14). For field efficiency to be confirmed in terms of potential disease control, BCAs must account for low AUDPC and FD%. Area under the disease progress curve and FD% (X_1/X_0), in both trials had a positive correlation, with the 2002 trial accounting for the greatest correlation. Of the two quantitative methods, AUDPC is considered the more accurate method of determining disease progress, as initial disease (X_o) may be variable for the different plots. Final percentage disease is also based on only one final disease rating. However, the good correlation between AUDPC and FD% does suggest that FD% could be used for future disease determination where only one rating, versus at least five for AUDPC, would be required. The correlation would require further verification to determine its stability.

Disease control differences between treated and untreated plots were found to be significant for the 2000 trial only. PUNCH XTRA was the best treatment, differing significantly from BIOSTART and the control. ROOTSHIELD showed no significant difference in disease control achieved with PUNCH XTRA and could, therefore, be considered as an alternative to PUNCH XTRA. ROOTSHIELD, however, did not differ significantly from the zero control treatment in terms of AUDPC and therefore would not provide efficient control. In the 2002 trial, *Trichoderma* kd differed significantly from the control in terms of AUDPC. Although ROOTSHIELD and *Trichoderma* kd are both *T.*

harzianum-based treatments, differences could be attributed to the different isolates, formulations (carrier) used and environmental conditions (Handelsman and Stabb, 1996) during the trial periods. Significant differences shown here do suggest the potential for isolates of *T. harzianum* as an alternative to chemical disease control.

Statistical differences were also shown for the treatment doses of the 2000 trial. The significant difference between the full dosage rate and the control for PUNCH XTRA, rendered the manufacturer's application rate (i.e. full dose treatment) correct. To be considered as an alternative to fungicides, microbial treatments must show no difference in terms of disease control against the fungicide. The full rate of ROOTSHIELD would thus be the correct dosage rate to use, as the half rate of ROOTSHIELD differed significantly from that of the full rate of PUNCH XTRA.

Differences between treatment type doses were also determined by regression analysis and linear fit curves. Treatment type doses over both trials, except full dosage rate of *Trichoderma* kd for the 2002 trial, resulted in a decrease in disease. However, a decrease in disease was also true for zero dosage treatments. This may be attributed to the changing of seasons (autumn) which was noted to decrease disease incidence. The gradients (m) of the linear fit for dosage rates were compared using a simple "t" test, where t = m/SE; (m) with (n-2)df. The calculated t values were, however, all less than the tabulated values at 95% F probability, gradient differences thus being non-significant ($P \leq 0.05$).

Although non-significant, linear fit curves showed the trend of greater disease control associated with half and full doses of treatment types. Discrepancies were, however, associated with the full rate of ROOTSHIELD and the half rate of BIOSTART. Dosage rates are important considerations, as biocontrol efficiency is likely to increase with enhanced growth and establishment of the BCA (Dandurand and Knudsen, 1993). Both bacteria and fungi will multiply rapidly within a conducive environment. Low disease control associated with the full dosage rate of ROOTSHIELD may be attributed to the antagonist becoming pathogenic at a high population level (Handelsman and Stubb,

1996). If the carrying capacity has been exceeded and the system cannot sustain the antagonist, the dying antagonists may fuel the prolification of soil pathogens (Dandurand and Knudsen, 1993).

Bipolaris sp. are also associated with root infections and root rots (Smiley *et al.,* 1992). Antagonistic microbes are known to colonize the rhizosphere, replacing the existing microflora and forging a close relationship with the plant root and growth-promotion responses (Harman, 2000). Potential growth stimulation was determined in terms of dry and wet weights. Dry matter percentage gives an overall assessment of growth, accounting for both foliage and root growth. The aim associated with assessing plant growth was to establish if disease control, achieved using BCAs, accounted for increased plant growth. This was based on the assumption that the correlation between root and shoot growth is equal. However, the correlation between wet and dry weight, for the 2000 trial was negative, while for the 2002 trial it was positive. Due to this variability no conclusion could be drawn.

ROOTSHIELD, according to the manufacturers, provides season-long control of root diseases, as *T. harzianum* colonises the entire root surface. Although root colonization was not determined, it was assumed that the microbes would have colonized the thatch layer, the soil and the plant rhizosphere. This renders the root system healthier and stronger, increasing the potential for water absorption (thus increased wet weight), nutrient uptake and soil exploration. A healthier plant being less vulnerable to disease, could explain low AUDPC and FD%'s associated with increased wet weight (negatively correlated over both trials). Increased plant wet weight could thus indicate control of pathogens in the rhizosphere. The increase in root growth may also be attributed to a rooting hormone (auxin) associated with *T. harzianum* (Harman, 2000). Plant-growth-promoting-rhizobacteria (such as *B. subtilis*) also increase plant growth via the production of plant growth regulation phytohormones (Schroth and Becker, 1990). In the 2002 trial, both the wet and dry weights showed a positive correlation, with a decrease in weights associated with increased disease incidence.

There are a number of factors which may account for the inconsistent results and non-significant differences observed in the trials. Inconsistency may be attributed to the treatments applied. Treatment between the two trials did vary except for the fungicide treatment. Correlation between PUNCH XTRA treatments was high (99%) and thus trial data variation was attributed to biocontrol treatments. The microbial formulations used for control of Helminthosporium leaf spot were rhizosphere treatments. Disease control thus will be attributed to successful colonization of the root zone and systemic acquired resistance (Handelsman and Stabb, 1996). Treatment applications were by means of a watering can, with BIOSTART applied using a pressurized spray bottle. Treatment applications were therefore limited to the foliage and thatch layers, with little of the treatment percolating into the soil profile to colonize the rhizosphere. Successful rhizosphere colonization results in disease control and plant growth stimulation (Dandurand and Knudsen, 1993; Kapulnick, 1996). However, colonization of the thatch layer will have resulted in reduced disease inoculum and thus a reduction in disease symptoms as was noted with microbial treatments in the linear fits of the disease progress curves.

Survival rates of antagonists after one day post inoculation *in vivo*, have been reported to be fewer than 1% (Leben, 1985). Reduced disease control with *Bacillus* B69 particularly, may be due to talcum powder residue left on the leaf surface, which was still visible a week after the application of the treatment (Figure 4.14). A leaf surface is an adverse environment to any microbe (Blakeman, 1985). Bacterial spores may also have become "trapped" by the talcum powder.

Figure 4.14 White talcum powder residue associated with the application of *Bacillus* B69 for Trial 2002 conducted at Cedara. The talcum powder acts as a carrier agent for *Bacillus subtilis* (Cedara, 2002).

Variability in results obtained between the two trials may not only be attributed to the different treatment used by also that FD% indicated a higher disease pressure for the 2000 trial. This could attribute for the non-significant treatment differences (types and doses) noted for the 2002 season. Insignificant differences between treatment types and treatment doses (for the 2000 and 2002 trials) could be attributed to the rating scale used (Figure 4.1) Although the use of predetermined rating scales to determine the percentage plant material infected is useful for standardizing results, it is often difficult to place the diseased plants into a specific rating class. A further disadvantage is that the sample units for each rating set were based on random plants within the trial plots. This would be overcome by means of increasing the sample size. An increased sample size would also "even out" rating class discrepancies.

Non-significance results in the 2002 trial may be attributed to the reduction in the number of replicates from 5 to 4 per treatment. This is reiterated by the standard error and CV%'s for analysis of variance for the 2002 trial being higher than for the 2000 trial. Standard error and CV% could have been reduced by reducing the number of treatments, i.e., implementing only one single zero control treatment across all treatment type doses. The effect of this would be an increased number of replicates per treatment which, in turn would decrease standard error. Alternatively, increasing plot sizes would also reduce CV% (Rayner, 1969). In terms of the treatments applied, the use of different BCAs for trials may have resulted in the differences in disease control acheived. Increased disease incidence for the 2000 trial may have been associated with the higher rainfall received during February to April 2000 (82.03mm difference between the trials).

For assessment of fertilizer rates, soil types or grazing habits the use of blocked treatment designed trials are useful (Stevens, 2002)[4]. However, implementation of a complete randomized block design makes for easier data analysis and accounts for less biased observations. The trial design implemented for the 2000 trial, followed a randomization of treatment types (i.e., ROOTSHIELD, BIOSTART and PUNCH XTRA) but a patterned dosage rate within the treatment blocks (i.e., full, half and zero; full, half and zero etc). This made for easier application of treatments, but may have biased dosage rate effects on plant growth due to inter-treatment interactions. To eliminate this interaction, however, 0.5m borders surrounded the individual treatment plots and the sample area was restricted to 40cm from the treatment plot border. In terms of treatment type, this block design of treatment type doses will reduce potential treatment drift during application. This was likely, as treatment plots measured only $1m^2$.

[4] Stevens, K. 2002. Cedara Biometrician. Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200, South Africa. Tel: (+27) 33 3559449

Non-significant results may also be attributed to instead of one single zero treatment control over all treatment types, a control treatment was included for each treatment type. To eliminate this treatment error, data was analysed for any potential trends by means of calculating a cumulative mean over all treatment types for the zero dosage rates. Zero treatments therefore had three times the number of experimental plots than the full and half treatments, the uneven number of plots increasing standard error and CV%. Using a single zero control treatment across all treatments for the 2000 trial (Table 4.7), thus eliminating the treatment error in having three different values for the same treatment, rendered significant differences. High CV%'s (>20%) were however noted, questioning the validity of significant differences noted. High CV% may be attributed to the difference in replicated plots between the treated plots (50 plots each) and the mean zero control plots (3 x 50 =150 plots in total). In general, one would expect a CV% of approximately 20% with plots measuring $1m^2$ (Rayner, 1969).

Concluding remarks

The application of biocontrol microbes and the survival of these microbes restores the "biological balance" that should exist between antagonistic and pathogenic microbes, but has been disrupted by agricultural practices. To optimise microbe efficiency and establishment in a specific niche, microbial characteristics, environmental and resource requirements, as well as the proposed mechanisms of activity need to be established through research. A lack of understanding of some of these factors may have attributed to non-significant results obtained in the trials. It was, however, concluded that BCAs increased disease control. However, variability in results does limit the implementation of biocontrol The effect of increased disease control being attributed to increased plant growth, in terms of dry matter percentage, was also variable. It is important to bear in mind that microbial treatments are "living" and therefore may not always be as effective as the manufacturers state.

4.4 REFERENCES

Agrios, G. 1997. Plant Pathology, 4th edition. Academic Press, California: United States of America.

Alcorn, J.L. 1988. The Taxonomy of *"Helminthosporium"* species. Annual Review of Phytopathology **26:** 37-56.

Anon, 2000. Genstat for Windows. Release 4.2, 5th edition. VSN International Ltd, Oxford: United Kingdom.

Baker, C.J., J.R. Stavely and N. Mock. 1985. Biocontrol of bean rust by *Bacillus subtilis* under field conditions. Plant Disease **69:** 770-772.

Blakeman, J.P. 1985. Biological succession of leaf surface microorganisms in relation to biological control. In: C.E. Windels and S.E. Lindow (eds.). Biological control on the phylloplane. American Phytopathology Society, Minnesota: United States of America. p. 6-30.

Camp, K.G.T. 1995. The bioresource groups of KwaZulu-Natal. Cedara Report N/A/95/32. KwaZulu-Natal Department of Agriculture, Pietermaritzburg: South Africa.

Couch, H.B. 1995. Diseases of turfgrasses, 3rd edition. Krieger Publishing, Florida: United States of America.

Dandurand, L.M. and G.R. Knudsen. 1993. Influence of *Pseudomonas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma harzianum* in the spermosphere and rhizosphere of the pea. Phytopathology **83:** 265-270.

Deacon, J.W. and L.A. Berry. 1992. Modes of action of mycoparasites in relation to biocontrol of soilborne plant pathogens. In: E.C. Tjomas; G.C. Papavizas and R.J.Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 157-164.

Froyd, J.D. 1997. Can synthetic pesticides be replaced with biologically-based alternatives? Journal of Industrial Microbiology and Biotechnology **19:**192-195.

Guetsky, R., D. Shteinberg, Y. Elad and A. Dinoor. 2001. Combining biocontrol agents to reduce the variability of biological control. Phytopathology **91:** 621-627.

Hall, A.S. 1991. A study of pasture diseases in Natal. MSc. Thesis. University of Natal, Pietermaritzburg: South Africa.

Handelsman, J. and E.V. Stubb. 1996. Biocontrol of soilborne plant pathogens. The Plant Cell **8:** 1855-1869.

Harman, G.E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease **84:** 377-393.

Harman. G.E. 2001. *Trichoderma* spp., including *T. harzianum, T. viride, T. koningii, T. hamatum* and other spp. Cornell University: Geneva: United States of America. www.nysaes.cornell.edu/ent/biocontrol/pathogens/trichoderma.

Hughes, F.L. and F.H.J. Rijkenberg. 1985. Scanning electron microscopy of early infection in the uredial stage of *Puccinia sorghi* in *Zea mays.* Plant Pathology **34:** 61-68.

Inbar, J., M. Abramsky, D. Cohen and I.Chet. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings grown under commercial conditions. European Journal of Plant Pathology **100:** 337-346.

Kapulnik, Y. 1996. Plant growth promotion by rhizosphere bacteria. In: Y. Wisel, A. Eshel and U. Kafkafi (eds). Plant roots: the hidden half, 2nd edition. Marcel Dekker, New York: United States of America. p. 769-781.

Krebs, B., B. Höding, S. Kübart, M. AlemayehuWorkie, H. Junge, G. Sshiedeknecht, R. Grosch, H. Bochow and M. Hevesi. 1998. Use of *Bacillus subtilis* as a biocontrol agent.I. Activities and characterization of *Bacillus subtilis* strains. Journal of Plant Diseases and Protection **105:** 181-197.

Leben, C. 1985. Biological control strategies in the phylloplane. In: C.E. Windels and S.E. Lindow (eds.). Biological control on the phylloplane. American Phytopathology Society, Minnesota: United States of America. p. 1-5.

Lenné, J.M. 1994. Diseases of other pasture grasses. In: J.M. Lenné and P. Trutmann (eds.). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 169-194.

Lewis, J.A., R.P. Larkin and D.L. Rogers. 1998. A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in a soilless mix. Plant Disease **82:** 501-506.

Marrone, P.G. 1999. Microbial pesticides and natural products as alternatives. Outlook on Agriculture **28:**149-154.

Nel, A., M. Krause, N. Ramautar and K. van Zyl. 1999. A guide for the control of plant diseases. National Department of Agriculture, Pretoria: South Africa.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Annual Review of Phytopathology **23:** 23-54.

Rayner, A.A. 1969. A first course in biometry for agricultural students. University of Natal Press, Pietermaritzburg: South Africa.

Robinson, D.L., R. Thomson and P.G.N. Digby. 1982. REML - program for the analysis of non-orthogonal data by restricted maximum likelihood. Compstat 1982, Proceedings in Computational Statistics, Part II (supplement). Verlag: Vienna. p 231-232.

Schroth, M.N. and J.O. Becker. 1990. Concepts of ecological and physiological activities of rhizobacteria related to biological control and plant growth promotion. In: D. Hornby (ed). Biological control of soil-borne plant pathogens. CAB International, Wallingford: United Kingdom. p. 389-414.

Schroeder, C.B. and H.B. Sprague. 1996. Turf management handbook, 5th edition. Interstate Publishers, Illinois: United States of America. p. 5-147, 150-155.

Smiley, R.W., P.H. Dernoeden and B.B. Clarke. 1992. Compendium of turfgrass diseases, 2nd edition. American Phytopathology Society, Minnesota: United States of America.

Smith, K.P. and R.M. Goodman. 1999. Host variation for interactions with beneficial plant-associated microbes. Annual Review of Phytopathology **37:** 473-491.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology **26:** 379-407.

Williamson, S. 1998. Understanding natural enemies; a review of training and information in the practical use of biological control. Biocontrol News and Information **19:** 117-125.

Yuen, G.Y. 2002. Personal communications, January 2002. gyuen@unl.edu

Zhang, Z. and G.Y. Yuen. 1999. Biological control of *Bipolaris sorokiniana* on tall fescue by *Stenotrophomonas maltophilia* strain C3. Phytopathology **89:** 817-822.

CHAPTER 5

POTENTIAL FOR GROWTH STIMULATION IN THE ESTABLISHMENT OF COOL SEASON TURFGRASS VARIETIES USING AMENDED BIOCONTROL AGENTS

ABSTRACT

Plant growth is influenced by a number of factors. To reduce environmental factors, growth stimulating microbes were tested *in vitro.* Pot trials comprising perennial (*Lolium perenne* L.) and annual ryegrass (*L. multiflorum* Lam.) were established within a greenhouse (°C and RH% controlled) at the University of Natal, Pietermaritzburg. The trials were to determine the potential of formulations of *Bacillus subtilis* Ehrenberg & Cohn. and *Trichoderma harzianum* Rifai for growth stimulation. Plant counts (%) and leaf measurements (mm) (6-20 days post seed inoculation) showed increased growth associated with microbial treatments. Increased leaf length of perennial ryegrass treatment the microbes was significantly ($P \leq 0.05$) different to the control treatment. The application of MICROBOOST® increased microbial activity in that root lengths of annual ryegrass were significantly ($LSD_{(0.05)} = 6.69$) greater than treatments with no MICROBOOST. Microbial efficiency was also determined *in vivo*, using experimental plots at Cedara ($29^0$32'S, $30^0$17'E) of perennial ryegrass, fescue (*Festuca rubra* L.) and bentgrass (*Agrostis stolonifera* L.). Formulations of growth stimulating microbes: *B. subtilis*, *T. harzianum* and *Gliocladium virens* J.H. Miller, J.E. Gidens, A.A. Foster & von Arx., were applied to seeds as a dusting at planting, and at 10 day intervals for 10 weeks after planting. Growth stimulation was measured as increased plant counts (%), as well as root and shoot lengths (mm). Growth responses between the two trials differed, as did responses for the duration of the trial. In terms of increased germination rate, microbial treatments, with the exception of *G. virens,* ranked highest for growth simulation. At trial termination, significant ($P \leq 0.05$) differences in the final plant count were noted. This

indicated the potential for increased establishment associated with the microbe-based treatments. Non-significant ($P \geq 0.05$) differences in growth at 6 days after planting, indicated that microbes had little effect on germination rates. Microbe-based treatments caused increased root and shoot lengths. Mean root and shoot lengths for the three grass types were significantly ($P \leq 0.05$) increased. MICROBOOST had no significant ($P \geq 0.05$) effect on microbial activity in terms of increased germination rates, but root and shoot lengths were significantly ($P \leq 0.05$) increased. Growth stimulation associated with the microbe-based treatments was also observed by increased weed growth.

5.1 INTRODUCTION

The concept of using biological control agents (BCAs) was first recognised when researchers discovered that supposedly healthy plants were stunted by the activity of a number of root pathogens (Baker, 1992). Over the years this has led to renewed interest in biological control, with various investigations on disease control and growth stimulation incited by antagonistic microorganisms. It is assumed that antagonistic microbes colonize the rhizosphere following inoculation forming a close relationship with the plant's roots (Schroth and Becker, 1990). Upon colonization these microorganisms may cause plant growth stimulation (Kapulnik, 1996; Harman 2000). Commonly documented as growth promoting BCAs are *Trichoderma harzianum* Rifai strains. These are known to increase germination, seedling emergence and establishment (Raviv *et al.*, 1998), as well as improve dry weight of shoots, stems and roots under both greenhouse and field conditions (Windham *et al.*, 1986; Kleifeld and Chet, 1992). Commercial application includes the amendment of planting media or seed treatments (Knudsen *et al.*, 1991) resulting in growth stimulation, as well as disease control of soilborne pathogens (Agrios, 1997).

The aim of these trials was to determine the growth stimulation potential of experimental formulations of antagonistic microbes on grass species both *in vitro* and *in vivo*. Growth stimulation was determined as an increase in germination or plant emergence,

establishment rates and increased root and shoot lengths. The potentially adverse effect of increased weed growth associated with microbial amendments was also determined. It is assumed that growth stimulation could be due to colonization of the plant's rhizosphere by antagonists resulting in increased moisture and nutrient uptake by the plant (Cook, 1990). Antagonist colonization of the rhizosphere also potentially protects against soil pathogens which would reduce plant growth potentials (Harman, 2000).

In terms of growth stimulation, microbial formulations were applied to cool season grasses: Perennial Prelude II ryegrass (*L. perenne* L.), Junior fescue (*F. rubra* L.) and Crenshaw bentgrass (*Agrostis stolonifera* L.). Although temperate grasses are not that widely used in KwaZulu-Natal for turf establishment, cool season grasses were chosen due to the trial period falling over winter. In KwaZulu-Natal, plants are exposed to limited moisture (averaging 85mm), extreme daily temperatures (6.0 -20.3^{0}C) and severe frost (441 chill units) during the winter months (Camp, 1995). Temperate grasses are able to maintain active growth during the winter months and are tolerant of severe frost. Of these, bentgrass is more widely used on golf greens, while ryegrass and fescue are used to oversow rugby fields (Tainton and Klug, 2002).

Due to the BCAs used being experimental formulations, the recommended application rates were tested. The method and rate of microbial application plays a vital role in the establishment and maintenance of the mended antagonistic population (Fravel, 1992).Too low a population would not stimulate growth, while too high a population would exceed the carrying capacity of the rhizosphere (Papavizas, 1985; Handelsman and Stabb, 1996). Microbial activity is also dependent on nutrient competition on the rhizosphere (Lo, 1998). The application of MICROBOOST, a microbial activator, was therefore also tested. The assumption was that MICROBOOST causes a large boost of the multiplication of the antagonist.

The future success of BCAs is dependent on the stability of antagonists once inoculated into an environment where fluctuating extremes will be experienced (Koch, 1999).

5.2 MATERIALS AND METHODS

5.2.1 *IN VITRO* POT TRIAL

Trial site

This trial was conducted in a temperature, moisture controlled greenhouse (fluctuating between 20-25^{0}C, RH 80%) at the University of Natal, Pietermaritzburg, South Africa.

Trial design

The trial designs were that of randomised complete block design for each grass type, with six treatments and three replicates per grass type. Due to limited space, only ryegrass was established. This included Perennial Prelude II from Mayford Seeds[1] and Annual Exalta ryegrass obtained from McDonald Seeds[2].

Trial establishment

Into each 180mm diameter pot, containing a sterilized 50:50 mixture of perlite and silica sand, 10 seeds per grass type were planted. Eighteen pots were planted to perennial and annual ryegrass, respectively.

1 Mayford Seeds, P.O. Box 160, Lanseria, 1748, South Africa. Tel: (+27) 11 701 3335

2 McDonald Seeds, P.O. Box 40, Mkondeni, 3200, South Africa. Tel (+27) 33 3460121

Treatments

The antagonistic potential of experimental formulations comprising spore suspensions in inert carrier media, were determined against a water control. The treatments included *Bacillus subtilis* Ehrenberg & Cohn B69 in talcum powder (designated *Bacillus* B69) and *Trichoderma harzianum* Rifai kd in shredded wheat (designated *Trichoderma* kd) and *Gliocladium virens* Miller, Gidens, Foster & von Arx in kaolin with oak husk (designated *Gliocladium*). The products were obtained from Plant Health Products cc[3]. The product based on *Bacillus* B69 is still undergoing performance tests before commercial release.

The necessity of a starter nutrient formulation was also investigated. MICROBOOST is marketed by Microbial Solutions[4] as a microbial activator and was added at a rate of 0.25g/250mℓ to treatments *Bacillus* B69, *Trichoderma* kd and water. Control treatments were these treatments without the addition of MICROBOOST.

Initial treatments were applied at planting as a drench over the planted seeds. Treatments were premixed to a paste to which 250mℓ water was added. This was then poured over the pots, using a 5ℓ watering can, (one per treatment) with a pouring nozzle head of 6.2cm X 7.8cm, with spray holes 1mm apart. Treatments were reapplied 10 days after planting (DAP). Application rates used are shown in Table 5.1.

[3] Plant Health Products cc., P.O. Box 207, Nottingham Road, 3280, South Africa. Tel: (+27) 33 263 6130

[4] Microbial Solutions (Pty) Ltd., P.O. Box 103, Kya Sand, 2163, South Africa. Tel: (+27) 11 462-2408

Table 5.1. Application rates for treatments administered of *in vitro* pot trials, to determine treatment effects on germination rates and growth stimulation of annual and perennial ryegrass (2001)

Treatment number	Treatment name	Application rate administered	Manufacturer's application rate
1	*Bacillus* B69	0.25g/0.25ℓ	1g/1ℓ
2	*Bacillus* B 69 + MICROBOOST	0.25g:0.25g/0.25ℓ	
3	*Trichoderma* kd	0.25g/0.25ℓ	
4	*Trichoderma* kd + MICROBOOST	0.25g:0.25g/0.25ℓ	
5	Water	0.25ℓ	-
6	Water + MICROBOOST	0.25g/0.25ℓ	

Observations and analyses

Germination rates

Six DAP, germination percentage (%) was determined from each pot. This was repeated at 10, 13, 16 and 20 DAP.

Shoot and root growth

Leaf length (mm) per germinated plant in each pot was measured at 10, 13,16 and 20 DAP. Plants were removed from pots at 20 DAP. Shoots (aerial growth) were severed from roots and the roots washed to remove excess growing medium. Root and shoot samples were oven dried at 60°C for 48 hrs and dry weight (DW) determined.

Analysis

Data was statistically analysed using analysis of variance (ANOVA) in Genstat 5 (Anon, 2000).

5.2.2 *IN VIVO* FIELD TRIAL

Only perennial ryegrass was used to investigate growth stimulation *in vivo*.

Trial sites

A replicated field trial was conducted at Cedara (Department of Agriculture and Environmental Affairs), situated in the KwaZulu-Natal Midlands approximately 32km inland from Pietermaritzburg. The soil was a well-drained, deep sandy-clay Hutton form (MacVicar, 1991).

Trial design

Trial design was randomised for three grass types, with eight treatments and four replicates, laid out into 64, $1m^2$ plots.

Site preparation and establishment

Six weeks prior to the commencement of the trial, a representative soil sample (topsoil), as outlined by Miles (1991), was taken from each of the 16 small plots comprising the trial. A representative sample was submitted to the Soil Analysis and Fertilizer Advisory Service[5] for analysis, in terms of soil acidity and fertility status. The results showed soils with a permissible acid saturation less than 20%, thus no liming was required.

Phosphorus (P) in the form superphosphate at a rate of 2kg $96m^{-2}$ (210kg P ha^{-1}) and nitrogen (N) at a rate of 18kg N ha^{-1}, were applied to the trial area two days before planting. Potassium (K) was applied due to the removal of foliage (Miles, 1991). This was applied as potash at a rate of 2.9kg $96m^{-2}$ (300kg K ha^{-1}).

Fertilizer application coincided with pre-emergent weed control, consisted of the establishment of a fine tilth achieved via conventional land preparation. This was achieved using a 1.5m wide disc and harrow, to a depth of 150mm.

[5] Soil Analysis and Fertilizer Advisory Service, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200. Tel: (+27) 343371 ext 321

Once the new weed flush was 50mm high (three weeks later), the area was disced again to a depth of 150mm, together with the application of fertilizers (as mentioned above). The area was then harrowed and rolled with a Cambridge roller in preparation for planting. Weeds emerging after this time, were sprayed with 2,4-D for broad-leaf weed control.

Perennial Prelude II ryegrass and Junior fescue were hand-planted at a seeding rate of 5g $1m^{-2}$ (50kg ha^{-1}), to a depth of 12mm. Crenshaw bentgrass was planted at a seeding rate of 3g $1m^{-2}$ (30kg ha^{-1}), to a depth of 3mm because of the fine seeds. Seed was obtained from Mayford Seeds[1]. To ensure even coverage and distribution of the seed and microbes, seeds were divided into two. One half was sown in one direction and the other half at right angles to the first.

The first trial (Turf 1) was planted on 14 February 2001 and ran until April 2001. A second trial (Turf 2) was established, adjacent to Turf 1, on 5 March 2001 running until 1 June 2001 using the same trial design.

Treatments

The antagonistic potential of experimental formulations comprising spore suspensions in inert carrier media, were determined against a water control. The treatments included *Bacillus* B69 in talcum powder, *Trichoderma* kd in shredded and *Gliocladium virens* Miller, Gidens, Foster & von Arx in kaolin with oak husk (designated *Gliocladium*). The product formulations were obtained from Plant Health Products cc[3].

The necessity of a starter nutrient formulation was also investigated. MICROBOOST was added at a rate of 2g/4ℓ to treatments *Bacillus* B69, *Trichoderma* kd and water. Control treatments were these treatments without the addition of MICROBOOST.

The initial microbial treatments were applied at planting as a seed dusting of spore suspensions. The control comprised uninoculated seeds which were planted by hand into each plot, after which the plots were raked and lightly irrigated. The trial areas received

25mℓ water per week on a 72 hr cycle. Initial treatments were applied at planting, as a drench, using 5ℓ watering cans (one per treatment) with a pouring nozzle head of 6.2cm X 7.8cm, with spray holes 1mm apart. Treatments were reapplied at 10 day intervals over the 10 week trial period. Treatment application rates used are shown in Table 5.2.

Table 5.2. Application rates for microbial growth stimulation treatments, administered to *in vivo* turfgrass field trials, to determine treatment effects on germination rates and growth stimulation of perennial Prelude II ryegrass, Junior fescue and Crenshaw bentgrass, Cedara (2001)

Treatment number	Treatment name	Application rate administered	Manufacturer's application rate
1	*Bacillus* B 69	4g/4ℓ	1g/1ℓ
2	*Bacillus* B 69 + MICROBOOST	4g:2g/4ℓ	
3	*Trichoderma* kd	4g/4ℓ	
4	*Trichoderma* kd + MICROBOOST	4g:2g/4ℓ	
5	*Gliocladium*	4g/4ℓ	
6	*Gliocladium* + MICROBOOST	4g:2g/4ℓ	
7	Water	4ℓ	-
8	Water + MICROBOOST	2g/4ℓ	

Observations and analyses

Germination and establishment rates

Effects of the microbial treatments on germination rates were determined 10 DAP by plant counts from a 30cm diameter circle (Area = 706.9cm) of each $1m^2$ plots. Circular sample areas were confined to the center of the plots.

Circular germination counts were repeated for the Turf 2 trial. Diagonal counts were also recorded to determine a more representative in an attempt to determine which is better for determining establishment rates, the circular sample area or diagonal count. The diagonal was demarcated with a piece of string, and the number of plants (leaves) touching the measuring string were recorded. Diagonal counts extended from the same corner of the treatment plots for each count in an attempt to eliminate variability, as was observed for Turf 1.

Following a heavy downpour on 18 February (47.2mm), resulting in severe wash between treatment plots, Replicates 1 and 4 (edge replicates) were omitted after the second germination count. For Replicates 2 and 3 of Turf 1 and all replicates of Turf 2, germination counts and the re-application of treatments were done at approximately 10 day intervals over the 10 week trial period making up a total of seven repeated counts.

Shoot and root growth

From each plot, cores measuring 150mm deep by 50-80mm in diameter were removed. From the cores, the lengths of 20 shoots and roots were measured. Shoots were severed from the roots within a few hours of taking the soil cores and the length measured (mm). Roots and soil were allowed to dry for 24hrs at room temperature. Roots were then removed for measurement (mm). The total number of repeated cores removed was seven per treatment plot, over the 10 week trial period.

Weed growth

For each circular sample area and diagonal count, the number of weeds was also recorded, to determine the effect of microbial treatments on weed growth stimulation.

Analysis

All data was statistically analysed using analysis of variance (ANOVA) in Genstat 5 (Anon, 2000b).

5.3 RESULTS

5.3.1 *IN VITRO* TURFGRASS POT TRIALS

Germination rates

Tables 5.3 and 5.4 summarise the ANOVA tests for germination percentages (%). Linear fits to the germination percentages recorded over the trial period are graphically presented by Figures 5.1 and 5.2. Regression analysis of the germination curves over the trial periods revealed by low (<1) regression values (R_2). This shows the trends observed to be unlikely. Effect of the application of MICROBOOST to treatments is also presented in the above mentioned tables and figures.

For perennial ryegrass, 100% germination was observed for *Trichoderma* kd treatments. At 6 DAP, germination % differences between *Trichoderma* kd and *Bacillus* B69 -based treatments were significantly ($P \leq 0.05$) different to the control (Table 5.3).

Differences in growth stimulation observed for MICROBOOST applications were non-significant for perennial ryegrass ($P \geq 0.05$). In response to MICROBOOST applications, only *Trichoderma* kd appeared to have improved activity achieving 100% germination at 6 DAP. The *Trichoderma* kd treatment achieved this only at 16 DAP.

For annual ryegrass, *Bacillus* B69 accounted for greater germination rates in comparison to the control. All differences in growth stimulation observed were, however, non-significant ($P \geq 0.05$).

Treatments + MICROBOOST caused reduced microbial activity in terms of germination rates of annual ryegrass (Figure 5.2; Table 5.4). Reduced growth stimulation was, however, very small in comparison to the controls.

Perennial ryegrass appeared to respond better to treatments, the variation between counts was lower for perennial ryegrass. The high CV% for annual ryegrass (Figure 5.4) is unexplained, as growth stimulation with *Bacillus* B69 is almost 100% more than that of the water + MICROBOOST treatment, yet no significant difference was reflected.

Table 5.3 Table of means and ANOVA of perennial ryegrass (turf) germination rates, for treatments applied to *in vitro* pot trials (2001)

				GERMINATION PERCENTAGE				
		R[1]	R[2]	6 DAP	10 DAP	13 DAP	16 DAP	20 DAP
Treatments	Water	2	1	76.7	86.7	93.3	93.3	93.3
	Water + MICROBOOST	1		60	86.7	86.7	86.7	90
	Trichoderma kd	4	3	90	96.7	96.7	100	100
	Trichoderma kd + MICROBOOST	6		100	100	100	100	100
	Bacillus B69	5	2	96.7	96.7	96.7	96.7	96.7
	Bacillus B69 + MICROBOOST	3		90	93.3	93.3	93.3	93.3
Grand mean				85.6	93.3	94.4	95	95.6
LSD $_{(0.05)}$	Microbial treatments			20.4	10.8	10.9	10.5	9.9
Degrees of freedom (residual)				10				
F probability (P)	Microbial treatments			0.03 *	0.09 (NS)	0.27 (NS)	0.16 (NS)	0.21 (NS)
	MICROBOOST			0.57 (NS)	1.00 (NS)	0.59 (NS)	0.41 (NS)	0.55 (NS)
	Treatments + MICROBOOST			0.38 (NS)	0.79 (NS)	0.60 (NS)	0.78 (NS)	0.91 (NS)
Standard error (s.e.)				15.9	8.4	8.5	8.2	7.7
CV%				18.6	9	9	8.6	8

DAP = days after planting

R^1 = ranking between treatments over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.4 Table of means and ANOVA of annual ryegrass (pasture) germination rates, for treatments applied to *in vitro* pot trials (2001)

				GERMINATION PERCENTAGE				
		R^1	R^2	6 DAP	10 DAP	13 DAP	16 DAP	20 DAP
Treatments	Water	**2**	**1**	46.7	66.7	66.7	66.7	66.7
	Water + MICROBOOST	**1**		50	56.7	56.7	56.7	56.7
	Trichoderma kd	**4**	**2**	63.3	80	80	80	80
	Trichoderma kd + MICROBOOST	**3**		60	70	70	73	76.7
	Bacillus B69	**6**	**3**	86.7	93.3	93.3	93.3	93.3
	Bacillus B69 + MICROBOOST	**5**		73.3	90	90	90	90
Grand mean				63	76	76	77	77
$LSD_{(0.05)}$				NS				
Degrees of freedom (residual)				10				
F probability (P)	Microbial treatments			0.17 (NS)	0.14 (NS)	0.14 (NS)	0.16 (NS)	0.18 (NS)
	MICROBOOST			0.73 (NS)	0.50 (NS)	0.50 (NS)	0.58 (NS)	0.66 (NS)
	Treatments + MICROBOOST			0.87 (NS)	0.96 (NS)	0.96 (NS)	0.97 (NS)	0.97 (NS)
Standard error (s.e.)				26.7	23.5	23.5	24.6	25.8
CV%				42.3	30.9	30.9	32	33.4

DAP = days after planting

R^1 = ranking between treatments over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

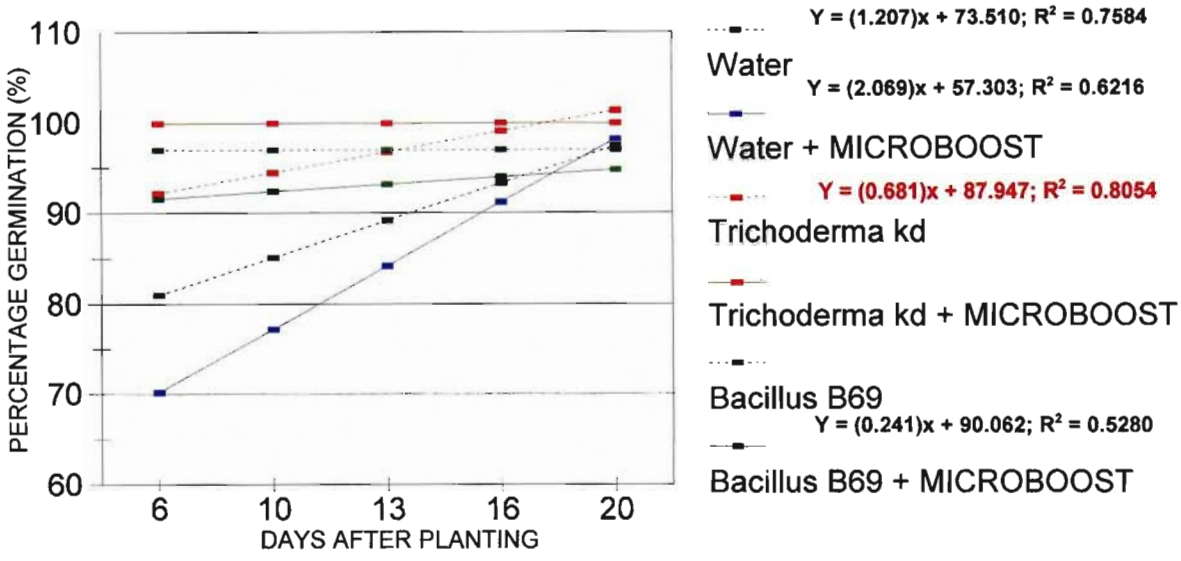


Figure 5.1 Linear fit of the germination percentages of Perennial Prelude 11 ryegrass (turf), as observed in the *in vitro* pot trial (2001).

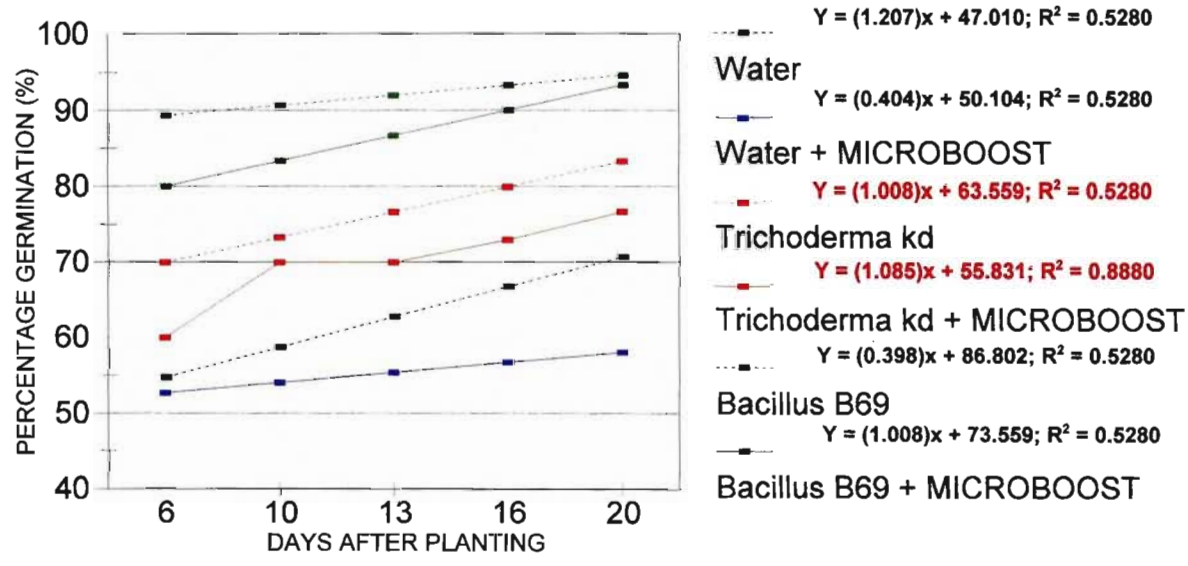


Figure 5.2 Linear fit of the germination percentages of Annual Exalta ryegrass (Pasture), as observed in the *in vitro* pot trial (2001).

Shoot lengths

Growth stimulation was also determined as increased shoot lengths over the trial period. (Tables 5.5 and 5.6). ANOVA tests showed significant ($P \leq 0.05$) differences within the microbial treatments for perennial ryegrass (turf) only (Table 5.5). Means for each treatment are presented in Figures 5.3 and 5.4.

Difference in shoot length between the *Bacillus* B69 treatments and the control was significant ($P \leq 0.05$) for perennial ryegrass only. The control treatments (mean of water and water + MICROBOOST) showed shorter shoot lengths in comparison to the microbial-based treatments for both perennial and annual ryegrass (Tables 5.5 and 5.6, Figures 5.3 and 5.4), suggesting growth stimulation with the use of microbial treatments. *Bacillus* B69 accounted for the longest shoot length of the grass types.

MICROBOOST applications for increased microbial activity were non-significant ($P \geq 0.05$) (Tables 5.5 and 5.6). However, the control + MICROBOOST caused greater shoot lengths in comparison to the control alone, for both perennial and annual ryegrass. *Trichoderma* kd + MICROBOOST accounted for not only 100% germination of perennial ryegrass (Table 5.3) but also greater shoot length. All other treatments + MICROBOOST caused decreased shoot lengths, in comparison to the microbial treatments alone.

Table 5.5 Table of means and ANOVA of perennial ryegrass (turf) shoot lengths, for treatments applied to *in vitro* pot trials (2001)

				LEAF LENGTH (mm)			
		R^1	R^2	10 DAP	13 DAP	16 DAP	20 DAP
Treatments	Water	**1**	**1**	38.8	43.3	60.9	73.5
	Water + MICROBOOST	**2**		44.5	49.4	65.8	76.6
	Trichoderma kd	**3**	**2**	47.4	51.4	68.4	78.2
	Trichoderma kd + MICROBOOST	**4**		46.8	51.2	71.6	83
	Bacillus B69	**6**	**3**	49.5	55.1	72.6	88.3
	Bacillus B69 + MICROBOOST	**5**		49.7	52.1	71.4	86
Grand mean				46.1	50.4	68.5	80.9
LSD $_{(0.05)}$	Microbial treatments			6.5	5.7	7.3	9.3
Degrees of freedom (residual)				10			
F probability (P)	Microbial treatments			0.05 *	0.05 *	0.06 (NS)	0.05 *
	MICROBOOST			0.47(NS)	0.65 (NS)	0.41 (NS)	0.60 (NS)
	Treatments + MICROBOOST			0.52(NS)	0.24 (NS)	0.64 (NS)	0.68 (NS)
Standard error (s.e.)				5.03	4.42	5.67	7.24
CV%				10.9	8.8	8.3	8.9

DAP = days after planting

R^1 = ranking between treatments over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.6 ANOVA of annual ryegrass (pasture) shoot lengths, for treatments applied to *in vitro* pot trials (2001)

				LEAF LENGTH (mm)			
		R^1	R^2	10 DAP	13 DAP	16 DAP	20 DAP
Treatments	Water	**2**	**1**	82	94.4	135	176.4
	Water + MICROBOOST	**1**		96.6	107.2	102.3	181
	Trichoderma kd	**5**	**2**	96.2	106.1	161.8	188.9
	Trichoderma kd + MICROBOOST	**4**		91.1	104.2	149.3	192.8
	Bacillus B69	**6**	**2**	94.8	109.5	162.6	206.3
	Bacillus B69 + MICROBOOST	**3**		86.8	94.6	154.2	181.7
Grand mean				91.2	102.7	144.2	187.8
$LSD_{(0.05)}$				NS			
Degrees of freedom (residual)				10			
F probability (P)	Microbial treatments			0.83(NS)	0.86 (NS)	0.24 (NS)	0.35 (NS)
	MICROBOOST			0.94(NS)	0.85 (NS)	0.39 (NS)	0.54 (NS)
	Treatments + MICROBOOST			0.28(NS)	0.29 (NS)	0.87 (NS)	0.33 (NS)
Standard error (s.e.)				12.45	14.22	21.31	18.09
CV%				13.6	13.9	13.9	9.6

DAP = days after planting

R^1 = ranking between treatments over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

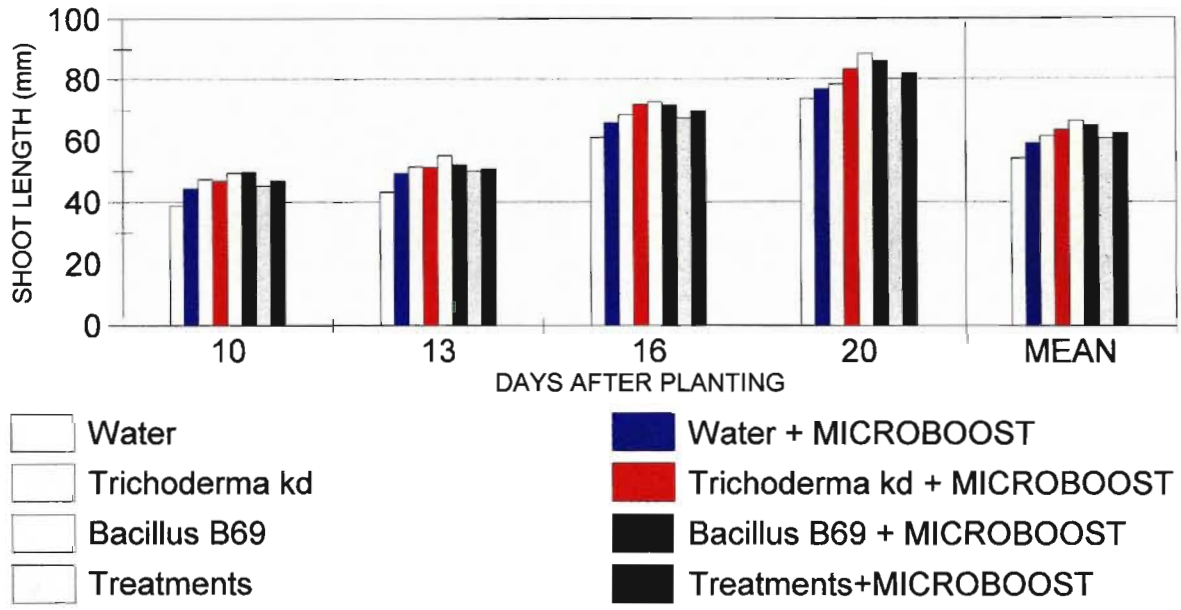


Figure 5.3 Effect of treatments on shoot length (mm) of Perennial Prelude II ryegrass (turf), as observed in the *in vitro* pot trial (2001).

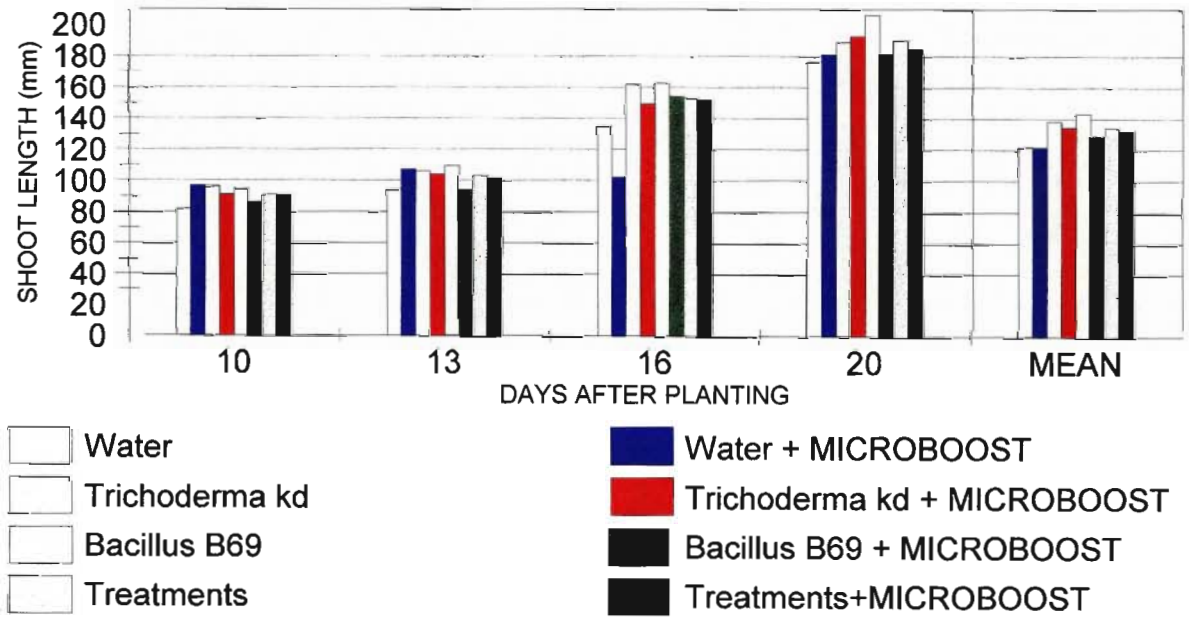


Figure 5.4 Effect of treatments on shoot length (mm) of Annual Exalta ryegrass (pasture), as observed in the *in vitro* pot trial (2001).

Shoot and root dry weights

Increased root and shoot DW was measured at trial termination (20 DAP) (Tables 5.7 and 5.8). The effect of microbial treatments on increased DW was non-significant ($P \geq 0.05$). Root, shoot and mean DW of the microbial treatments compared to the control (water and water + MICROBOOST), are graphically presented in Figures 5.5 and 5.6. Although non-significant , the water control accounted for the smallest growth increment. The amendment of MICROBOOST to the control (i.e., water + MICROBOOST) caused growth stimulation, such that the treatment showed the greatest root DW measured and the second greatest shoot DW (Figure 5.6). Increased DW was also shown for microbial + MICROBOOST treatments, with the exception of *Bacillus* B69 on annual ryegrass (Table 5.8, Figure 5.6). *Bacillus* B69 treatments accounted for greater DW than *Trichoderma* kd treatments (Tables 5.7 and 5.8).

Root and shoot dry weights for annual ryegrass (Table 5.8) showed a significant ($P \leq 0.05$) increase in root growth stimulation with the application of MICROBOOST. Increased root DW of *Trichoderma* kd + MICROBOOST and *Bacillus* B69 in comparison to the water control was also significant ($P \leq 0.05$). Low DW associated with *Trichoderma* kd was, however, non-significant ($LSD_{(0.05)}$ =3.2 and 11.6). Growth stimulation with the application of MICROBOOST for DW measurements of perennial ryegrass were non-significant (Table 5.7). Again, the high CV% is unexplained especially as *Trichoderma* kd + MICROBOOST shows almost a 100% higher DW than the control, yet microbial treatments showed no significant difference.

Table 5.7 Table of means and ANOVA of perennial ryegrass (turf) shoot and root dry weight, for treatments applied to *in vitro* pot trials (2001)

		DRY WEIGHT (g) AT 20 DAP					
		R^1	R^2	Shoots	R^1	R^2	Roots
Treatments	Water	**1**	**1**	4.46	**1**	**1**	11.4
	Water + MICROBOOST	**4**		4.94	**3**		16.2
	Trichoderma kd	**3**	**2**	4.86	**2**	**2**	12.8
	Trichoderma kd + MICROBOOST	**5**		5.5	**6**		19.9
	Bacillus B69	**2**	**3**	4.69	**4**	**3**	16.6
	Bacillus B69 + MICROBOOST	**6**		7.9	**5**		16.8
Grand mean		5.39			15.6		
$LSD_{(0.05)}$		NS					
Degrees of freedom (residual)		10					
F probability (P)	Microbial treatments	0.26 (NS)			0.57 (NS)		
	MICROBOOST	0.09 (NS)			0.12 (NS)		
	Treatments + MICROBOOST	0.30 (NS)			0.50 (NS)		
Standard error (s.e.)		1.59			5.03		
CV%		29.5			32.2		

DAP = days after planting

R^1 = ranking between treatments over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.8 Table of means and ANOVA of annual ryegrass (pasture) shoot and root dry weight, for treatments applied to *in vitro* pot trials (2001)

		DRY WEIGHT (g) AT 20 DAP					
		R^1	R^2	Shoots	R^1	R^2	Roots
Treatments	Water	**1**	**1**	8.41	**1**	**1**	13.7
	Water + MICROBOOST	**5**		11.42	**6**		34.3
	Trichoderma kd	**2**	**2**	9.19	**2**	**2**	19.4
	Trichoderma kd + MICROBOOST	**6**		11.7	**4**		26.2
	Bacillus B69	**4**	**3**	10.59	**5**	**3**	27.4
	Bacillus B69 + MICROBOOST	**3**		10.48	**3**		25.2
Grand mean				10.3			24.2
LSD $_{(0.05)}$	MICROBOOST			1.9			6.7
	Treatments + MICROBOOST			3.2			11.6
Degrees of freedom (residual)				10			10
F probability (P)	Microbial treatments			0.80 (NS)			0.63 (NS)
	MICROBOOST			0.06 (NS)			0.02 *
	Treatments + MICROBOOST			0.30 (NS)			0.04 *
Standard error (s.e.)				1.74			6.28
CV%				16.9			25.8

DAP = days after planting

R^1 = ranking between treatments over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

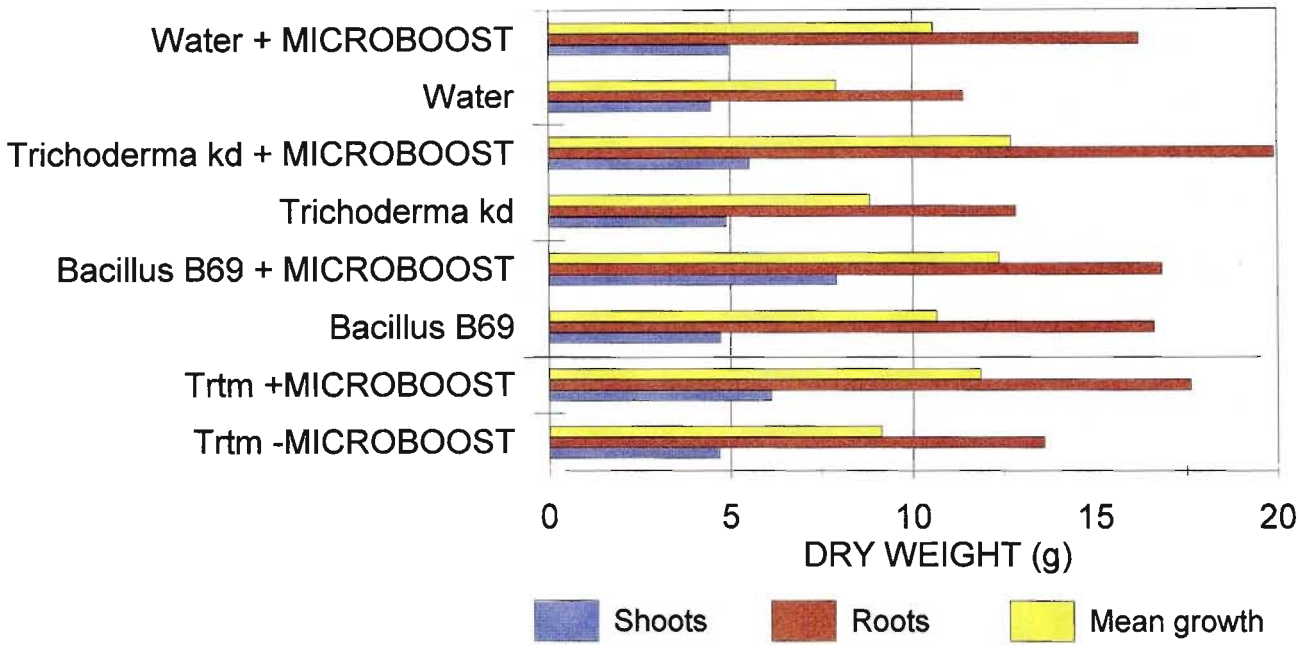


Figure 5.5 **Effect of treatments on growth stimulation determined by shoot and root dry weight (g) of perennial ryegrass (turf), as observed in the *in vitro* pot trial (2001).**

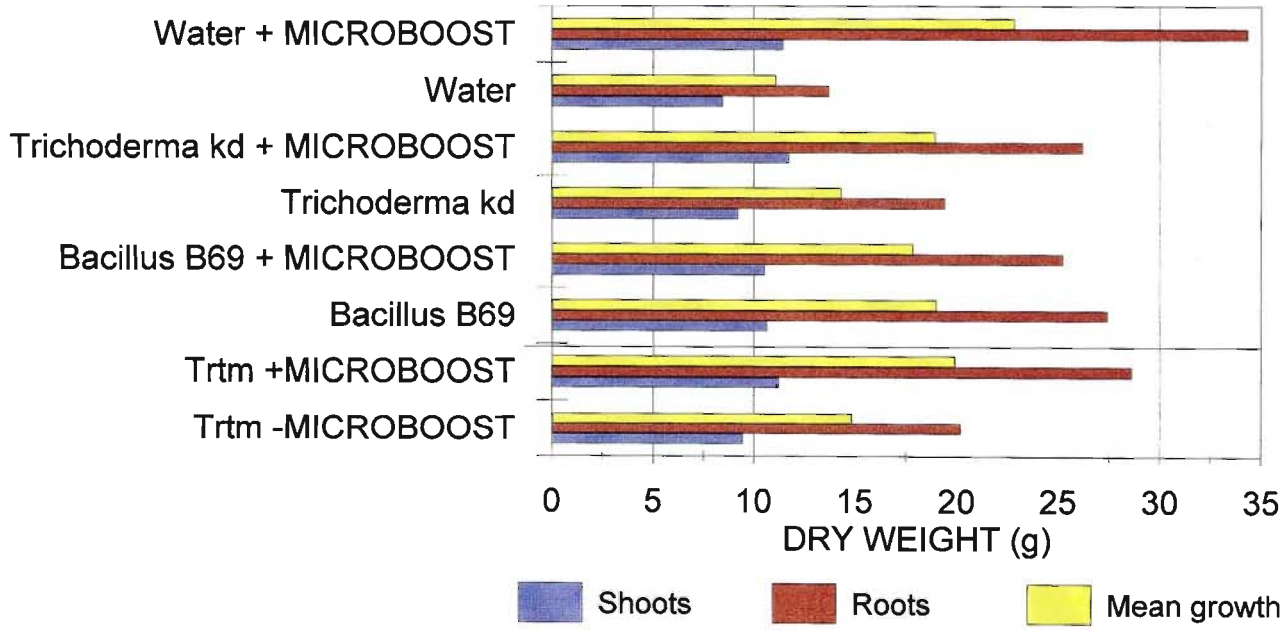


Figure 5.6 **Effect of treatments on growth stimulation determined by shoot and root dry weight (g) of annual ryegrass (pasture), as observed in the *in vitro* pot trial (2001).**

Mean growth was determined as a mean over root and shoot dry weights

5.3.2 *IN VIVO* FIELD TRIALS

Trends of growth stimulation were inconsistent over both trials for grass types and treatment applications.

Germination and establishment rates

Based on lower CV% and data distribution from residual plots, a representative area was best for determining plant counts. Diagonal counts accounted for very high CV% (>37.3%) even after log transformation.

Significance is summarised in ANOVA tables (Tables 5.9 and 5.10). In terms of initial germination, Turf 1 (Table 5.9) showed significant ($P \leq 0.05$) growth stimulation for *Bacillus* B69 and control treatments on ryegrass and bentgrass at 9 and 20 DAP. For Turf 2, significant ($P \leq 0.05$) differences between microbial treatments and the control were noted for 41 and 50 DAP on bentgrass only (Table 5.10). Significant deviations at 59 and 69 DAP, denoted significant growth stimulation differences between the microbial treatments for bentgrass only.

Plant counts for *Gliocaldium*, *Trichoderma* kd and *Bacillus* B69 treatments were significantly different ($P \leq 0.05$) (Table 5.10). However, variability between trials resulted in this being unlikely. Statistical analysis of the effect of MICROBOOST revealed no significant ($P \geq 0.05$) impact on increased microbial activity and plant growth stimulation.

Tables 5.9 and 5.10 also show treatment effects on germination and establishment rates of ryegrass, fescue and bentgrass for Turf 1 and 2 trials. Treatment means over the trial period were ranked, showing the effect of microbial treatments with and without MICROBOOST (R^1) (i.e., *Bacillus* B69 versus *Bacillus* B69 + MICROBOOST) and the effect of microbial treatment (mean of, for example, *Trichoderma* kd and *Trichoderma* kd + MICROBOOST) versus water-based treatments (R^2).

Treatments (R^1 from means tables) are also graphically presented for each grass type for plant counts over the trial period (Figures 5.7-5.9). A treatment mean over Turf 1 and 2 was determined for each grass type and this was ranked for the effect of treatments on increased plant coverage (Figures 5.7-5.9).

Establishment rates (growth stimulation) observed are shown in Figures 5.7-5.9. Treatment rankings (R^2) (Tables 5.9 and 5.10) showed that the overall microbe-based treatments accounted for reduced establishment rates in comparison to the water-based controls. However, water-based treatments accounted for lower establishment of fescue in Turf 1 (Table 5.9). But in comparison, on fescue in Turf 2 (Table 5.10) the water-based treatments accounted for significantly ($P \leq 0.05$) greater establishment.

At 69 DAP (last data collection) the difference between germination of the microbe-and water-based treatment plots was significantly different ($P \leq 0.05$) (Figures 5.10 and 5.11). Means for treatment effects over the three grasses, showed the water-based treatments to only Rank 1 (Figure 5.10) and 2 (Figure 5.11) (i.e., ranked low in comparison to the microbial treatments). Mean germination over Turf 1 and 2, show *Gliocladium* treatments to have lower growth stimulation than the control (Figure 5.12). This was non-significant.

In response to MICROBOOST applications, rankings (R^1) again revealed variable results between Turf 1 and 2. Therefore, a mean of the treatments with MICROBOOST and mean of treatments with no MICROBOOST was determined (Tables 5.9 and 5.10). The mean over Turf 1 and 2 for the grass types, showed that MICROBOOST did not improve establishment for bentgrass but did improve establishment of ryegrass. Fescue responded differently in Turf 1 and 2, so no conclusion could be drawn. Figure 5.12, summarises the effect of MICROBOOST over all three grass types. Overall, *Trichoderma* kd + MICROBOOST accounted for improved activity over *Trichoderma* kd alone. The application of MICROBOOST to the other treatments caused a reduction in emergence. Reduced microbial activity observed for the microbe-based treatments + MICROBOOST was, however, considered to be small and MICROBOOST effects on increased microbial activity was non-significant.

Table 5.9 **Table of means and ANOVAs of the representative germination counts of treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation of trial Turf 1, conducted at Cedara (Feb - April, 2001)**

Treatments (Trtm)	GERMINATION AND ESTABLISHMENT COUNTS																									
	RYEGRASS									FESCUE									BENTGRASS							
	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Water	7	3	37.7	50.5	51.5	56	62.5	65	70	3	1	18	32.5	40	44.5	50.5	49.5	67	4	2	10.3	21.5	49	62.5	75	78.5	85.5
Water + MICROBOOST	5		18.3	40.7	42	49	52.5	55	71	1		12.5	25.5	28	33	35	46.5	58.5	2		23	39.3	35.5	52.5	58	70	85
Trichoderma kd	3	4	16.3	23.3	30.5	42.5	47.5	65	84.5	2	2	18.8	30.5	26	41.5	44	60	79.5	5	1	20.3	30.5	47.5	52	71	82.5	93
Trichoderma kd + MICROBOOST	8		26.8	38.3	56	64.5	80	85	87	5		24.3	32.5	39	46	47	59.5	79.5	1		21.8	32	29	43	48	80	93
Gliocladium	6	2	9.7	30	37.5	48	65.5	70.5	79	8	4	21.3	32.2	38.5	54.5	67.5	78.5	80	3	3	27.8	39	50.5	51	52.5	68.5	90
Gliocladium + MICROBOOST	4		16.3	23.8	48	48	52	64	76	7		23	43.2	39.5	51	50	66.5	84.5	8		16.3	27.5	61	74.5	100	98.5	115
Bacillus B69	1	1	8.5	17	45	45.5	50	61	77.5	4	3	13.3	34.8	41.5	40.5	40	64.5	74	6	4	22.2	29	52.5	60	74	88.5	93.5
Bacillus B69 + MICROBOOST	2		15	34.5	36.5	41	46.5	64.5	68	6		21	29.5	46.5	50.5	51.5	56	78.5	7		34.5	44.5	57.5	63	77	90	99.5
Mean of Trtms with MICROBOOST	2		19.1	34.3	45.6	50.6	57.7	67.1	75.5	1		20.2	32.7	38.2	45.1	45.9	57.1	75.2	1		23.9	35.8	45.7	58.2	70.7	84.6	98.1
Mean of Trtms with NO MICROBOOST	1		18.1	30.2	41.1	48	56.4	65.4	77.7	2		17.8	32.5	36.5	45.2	50.5	63.1	75.1	2		20.1	30	49.9	56.4	68.1	79.5	90.5

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.9 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
LSD $_{(0.05)}$	Microbes	6.32	7.01	8.26	12.22	16.52	10.75	6.45
	Grass.Microbes	10.95	12.14	14.31	21.16	28.62	18.62	11.16
	Grass.Microbes.MICROBOOST	15.49	17.16	20.24	29.92	40.47	26.33	15.79
F probability (P)	Microbes	0.876 (NS)	0.695 (NS)	0.131 (NS)	0.740 (NS)	0.642 (NS)	0.071 (NS)	< 0.001 ***
	MICROBOOST	0.293 (NS)	0.179 (NS)	0.804 (NS)	0.730 (NS)	0.971 (NS)	0.937 (NS)	0.414 (NS)
	Grass.Microbes	0.012 **	0.024 *	0.293 (NS)	0.595 (NS)	0.776 (NS)	0.511 (NS)	0.240 (NS)
	Grass.MICROBOOST	0.882 (NS)	0.639 (NS)	0.457 (NS)	0.962 (NS)	0.856 (NS)	0.460 (NS)	0.184 (NS)
	Microbes.MICROBOOST	0.159 (NS)	0.345 (NS)	0.091 (NS)	0.504 (NS)	0.571 (NS)	0.614 (NS)	0.316 (NS)
	Grass.Microbes.MICROBOOST	0.071 (NS)	0.031 *	0.133 (NS)	0.575 (NS)	0.136 (NS)	0.325 (NS)	0.354 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		10.98	12.17	9.79	14.46	19.56	12.73	7.63
CV%		55.3	37.3	22.8	28.6	33.6	18.3	9.3

DAP = days after planting

Table 5.10 Table of means and ANOVAs of the representative germination counts of treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation of trial Turf 2, conducted at Cedara (March- May, 2001)

		GERMINATION AND ESTABLISHMENT COUNTS																								
		RYEGRASS									FESCUE									BENTGRASS								
		R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Treatments (Trtm)	Water	4	4	25.0	49.5	51.0	62.0	65.5	73.0	78.0	3	4	11.0	24.0	32.0	42.0	51.5	65.5	65.0	4	2	74.5	91.0	98.5	110.0	138.5	150.0	152.5
	Water + MICROBOOST	8		18.0	44.0	52.5	72.0	83.5	84.0	90.0	8		21.0	33.5	36.5	52.0	61.5	67.5	71.0	3		59.0	69.0	82.0	90.0	103.5	116.0	116.0
	Trichoderma kd	3	2	24.0	41.5	44.5	56.5	64.0	77.5	82.5	5	3	17.5	29.0	33.5	37.5	42.5	67.0	67.5	6	4	74.5	86.0	103.5	126.5	146.0	150.0	156.0
	Trichoderma kd + MICROBOOST	2		20.0	37.0	44.0	57.0	66.0	76.5	82.5	7		13.5	34.5	34.0	46.0	62.0	67.5	66.5	8		87.0	102.0	125.0	146.0	158.0	170.0	170.5
	Gliocladium	1	1	15.0	32.0	37.0	49.0	55.0	59.5	67.0	6	1	13.5	30.0	31.5	44.5	53.5	64.5	70.0	2	1	65.0	72.0	81.5	90.5	98.5	108.0	110.0
	Gliocladium + MICROBOOST	6		22.0	42.5	53.0	63.5	68.5	81.5	85.0	1		15.0	24.0	28.0	34.0	40.0	51.5	56.5	1		50.0	52.0	56.0	62.5	68.0	84.0	89.0
	Bacillus B69	7	3	24.0	43.5	52.0	60.5	73.0	90.5	94.0	4	2	18.5	32.0	37.0	41.5	40.5	56.0	67.0	7	3	58.5	80.5	110.5	144.5	148.0	153.0	155.0
	Bacillus B69 + MICROBOOST	5		28.5	47.5	49.0	57.0	63.0	80.0	85.0	2		15.0	33.0	33.5	35.5	38.0	54.5	57.5	5		67.5	80.5	105.0	132.5	139.0	145.0	146.0
	Mean of Trtms with MICROBOOST	2		22.1	42.7	49.6	62.4	70.2	80.5	85.6	2		16.1	31.2	33.5	41.9	50.4	60.2	62.9	1		65.9	75.9	92.0	107.8	117.1	128.8	130.4
	Mean of Trtm with NO MICROBOOST	1		22.0	41.6	46.1	57.0	64.4	75.1	80.4	1		15.1	28.7	33.0	41.4	47.0	63.2	67.4	2		68.1	82.4	98.5	117.9	132.8	140.3	143.3

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.10 cont....

		DAP 9	DAP 20	DAP 31	DAP 40	DAP 50	DAP 59	DAP 69
LSD $_{(0.05)}$	Microbes	10.32	12.94	16.61	18.63	19.21	18.25	17.97
	Grass.Microbes	17.87	22.42	28.78	32.27	33.27	31.61	31.12
F probability (P)	Microbes	0.341 (NS)	0.200 (NS)	0.163 (NS)	0.090 (NS)[a]	0.054 * [a]	0.033 * [a]	0.044 * [a]
	MICROBOOST	0.916 (NS)	0.830 (NS)	0.839 (NS)	0.826 (NS)	0.749 (NS)	0.630 (NS)	0.513 (NS)
	Grass.Microbes	0.523 (NS)	0.531 (NS)	0.290 (NS)	0.043 * [a]	0.052 * [a]	0.118 (NS)[a]	0.113 (NS)[a]
	Grass.MICROBOOST	0.927 (NS)	0.675 (NS)	0.772 (NS)	0.603 (NS)	0.360 (NS)	0.552 (NS)	0.489 (NS)
	Microbes.MICROBOOST	0.872 (NS)	0.754 (NS)	0.867 (NS)	0.750 (NS)	0.676 (NS)	0.850 (NS)	0.872 (NS)
	Grass.Microbes.MICROBOOST	0.489 (NS)	0.649 (NS)	0.780 (NS)	0.857 (NS)	0.767 (NS)	0.633 (NS)	0.697 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		12.22	15.33	19.67	22.06	22.75	21.61	21.27
CV%		35	30.4	33.5	30.9	28.3	23.7	22.4

DAP = days after planting

[a] = significant ($P \leq 0.05$) deviations on interactions noted

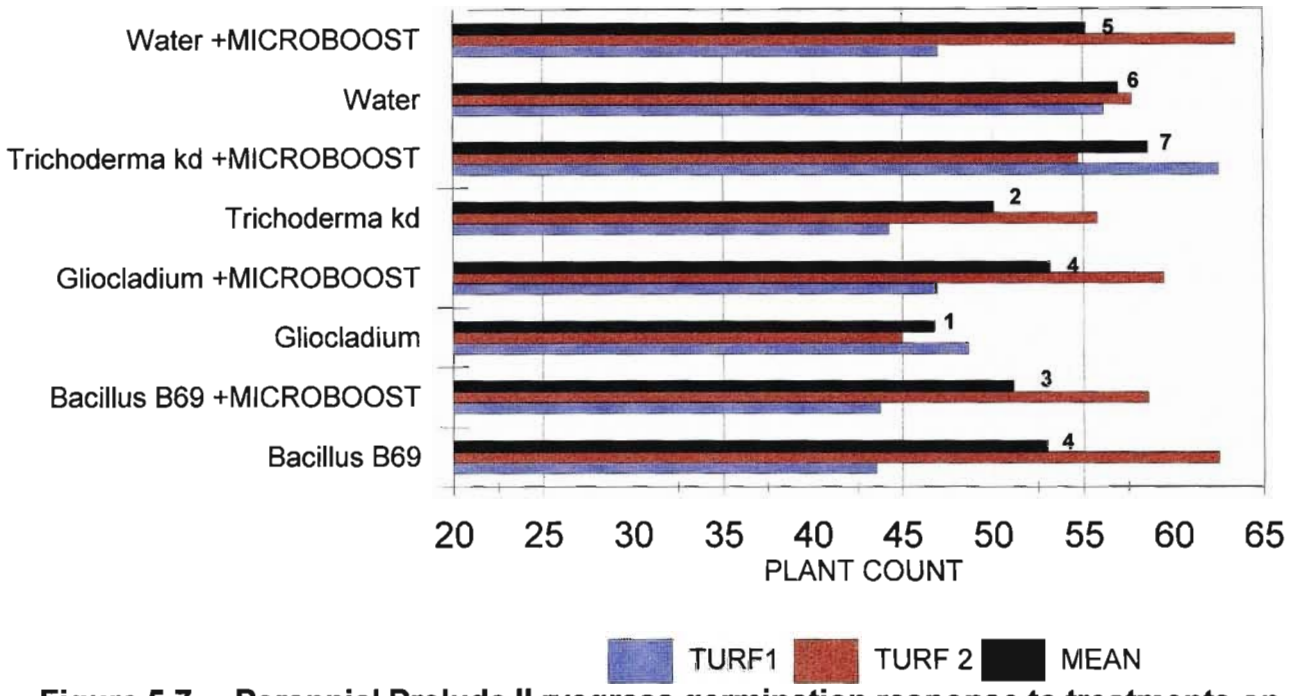


Figure 5.7 Perennial Prelude II ryegrass germination response to treatments on Turf 1 and 2, and a treatment mean over both *in vivo* turf trials, conducted at Cedara (2001).

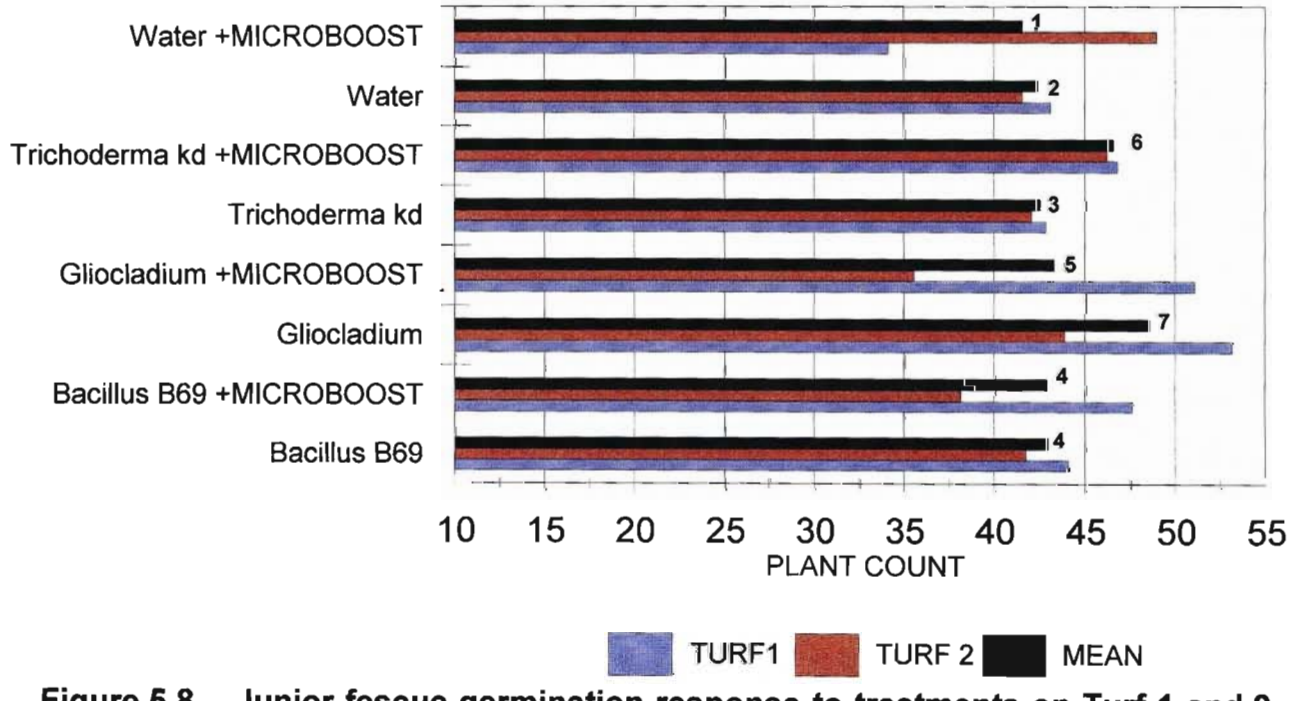


Figure 5.8 Junior fescue germination response to treatments on Turf 1 and 2, and a treatment mean over both *in vivo* turf trials, conducted at Cedara (2001).

1-7 = ranking of treatment effects on increased germination

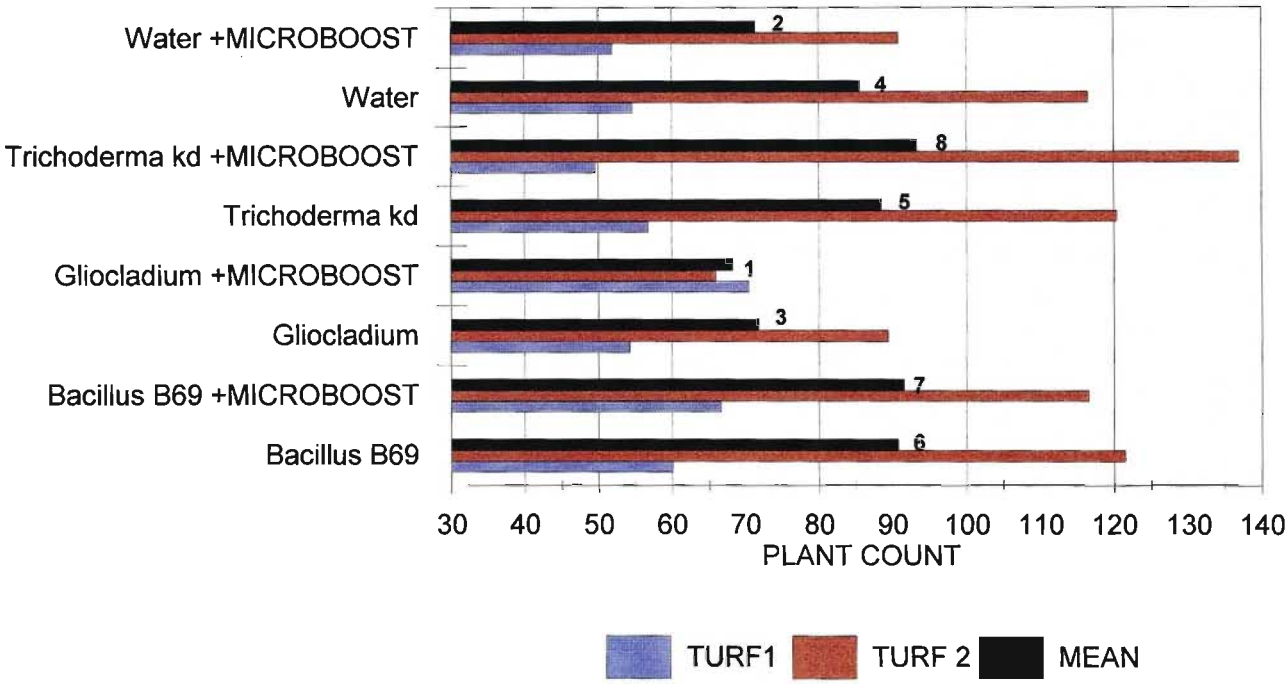


Figure 5.9 Crenshaw bentgrass germination response to treatments on Turf 1 and 2, and a treatment mean over both *in vivo* turf trials, conducted at Cedara (2001).

1-8 = ranking of treatment effects on increased germination

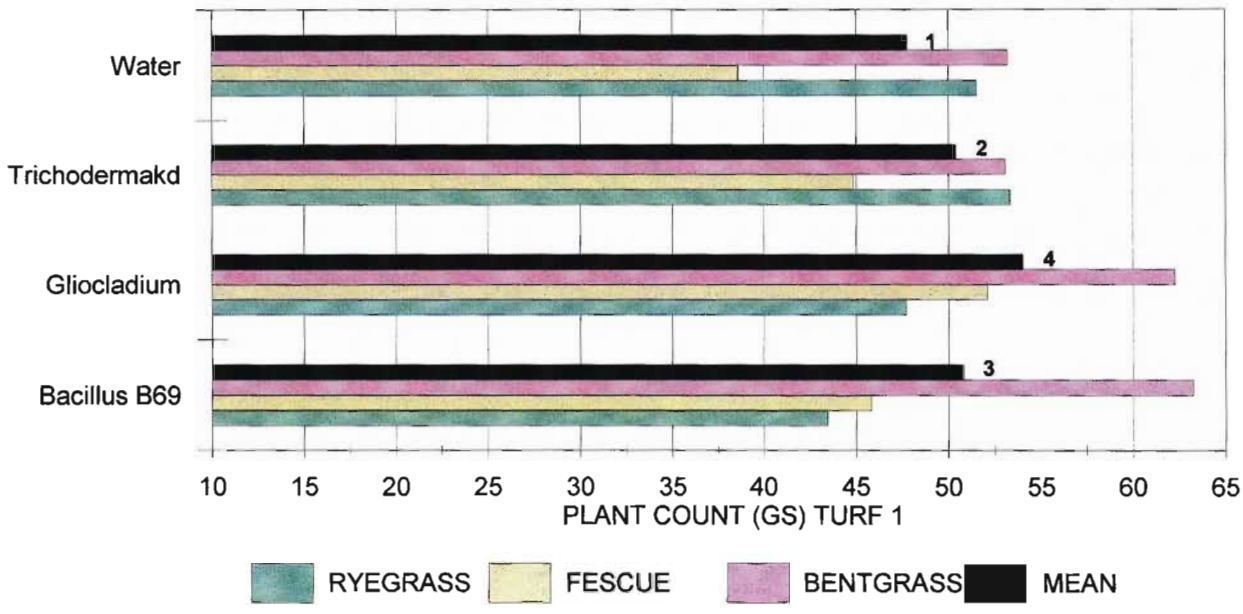


Figure 5.10 Comparison of microbe-based treatments versus the water-based controls for Turf 1 on perennial ryegrass, fescue, bentgrass and a mean over the three grass types. *In vivo* turf trials, conducted at Cedara (2001).

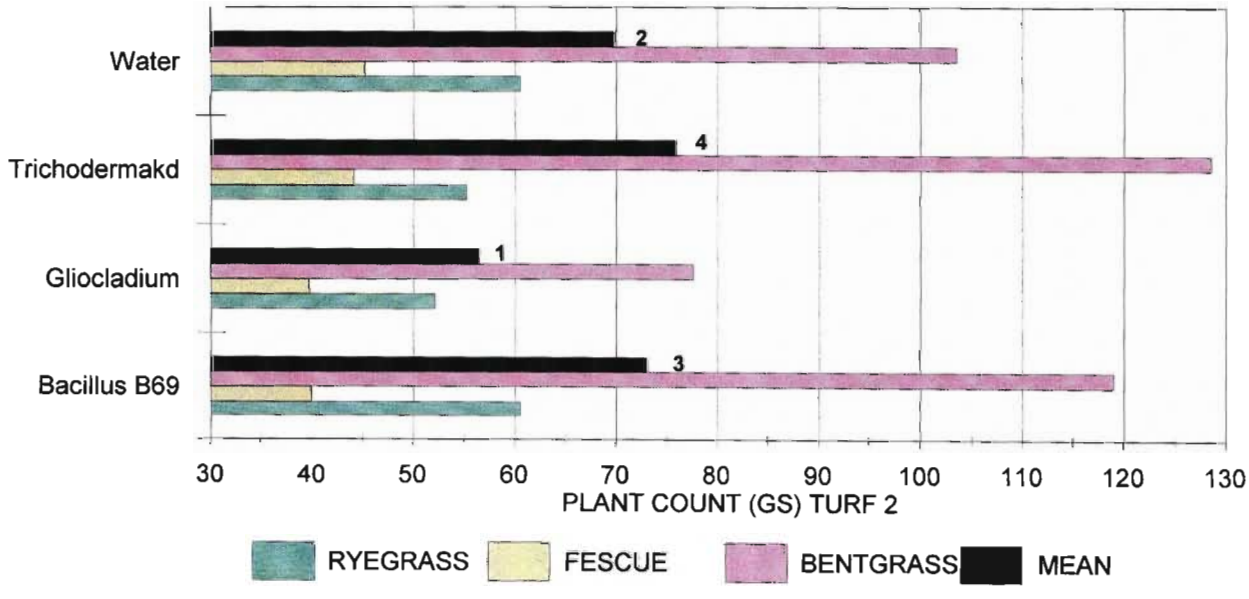


Figure 5.11 Comparison of microbe-based treatments versus the water-based controls for Turf 2 on perennial ryegrass, fescue, bentgrass and a mean over the three grass types. *In vivo* turf trials, conducted at Cedara (2001).

1-4 = ranking of the mean for control-based treatments and microbial-based treatments

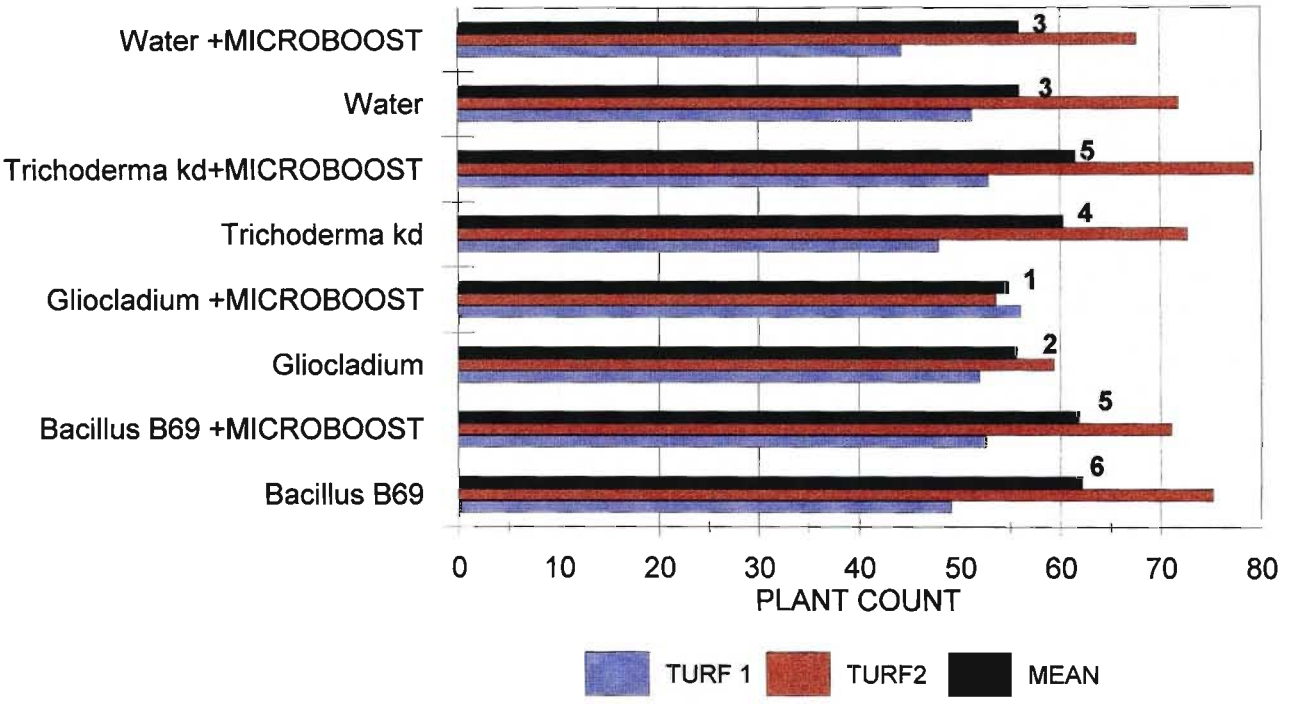


Figure 5.12 Effect of MICROBOOST for growth stimulation of the three grass types used in Turf 1 and 2, conducted at Cedara (2001).

1-6 = ranking of treatment effects on increased germination

Shoot and root growth

Tables 5.11- 5.14 show treatment effects on shoot and root lengths of ryegrass, fescue and bentgrass for Turf 1 and 2. Length differences between the control and microbe treatments were significant ($P \leq 0.05$). Significant differences between Turf 1 and 2 varied. However, significant differences were apparent for root and shoot lengths in both Turf 1 and 2 at 69 DAP. Treatments thus affected grass establishment rates significantly (exception being root length in Turf 1, Table 5.13 at 69 DAP). For Turf 2 significant differences were between microbe-based treatments and water-based ones. Highly significant differences were also noted between the microbial treatments (Table 5.12).

Treatment effects were ranked as means over the trial period showing treatment effects with and without MICROBOOST (R^1) and the effect of a mean for each microbe-based treatment versus the water-based controls (R^2). Treatment rankings showed the water-based treatments to account for shorter lengths (shoot and root). Extremes were apparent, such as root length of bentgrass treated with Water + MICROBOOST in Turf 1 which ranked 6 (Table 5.13).

Treatment effects are presented for each grass type for shoot (Figures 5.13-5.15) and root lengths (Figures 5.16-5.18). A treatment mean over Turf 1 and 2 was determined for each grass type. This was ranked for treatment effect on increased growth.

Figures 5.19 and 5.20 show the ranking of mean shoot and root length over Turf 1 and 2 for microbe- versus water-based treatments (R^2) for each grass type, as well as a mean over the three grass types. From these figures, microbial treatments rank higher than the water controls. For shoot length *Trichoderma* kd -based treatments ranked higher (Figure 5.19), but for root lengths *Gliocladium*-based treatments ranked highest. Growth stimulation of the individual grass types, i.e., ryegrass, fescue and bentgrass for Turf 1 and 2, varied from the mean (Figures 5.19 and 5.20). For shoot length of bentgrass in Turf 1, *Gliocladium*-based treatments ranked highest (Table 5.11). For root lengths of fescue in Turf 1 (Table 5.11) and ryegrass in Turf 2, both *Trichoderma* kd and *Bacillus* B69 treatments ranked higher than *Gliocladium* (Table 5.12). Reduced growth being significant for *Gliocladium* applications to ryegrass in Turf 2.

In response to MICROBOOST applications, Tables 5.11-5.14 show the effect of MICROBOOST on microbial activity for increased shoot and root length of the three grass types. MICROBOOST effects were inconsistent for the treatment types (including the control), grass types and Turf 1 and 2.

The effect of MICROBOOST for shoot length of the grass types for turf 1 were non-significant. Comparing (R^1) of MICROBOOST treatments, reduced shoot growth was associated with MICROBOOST applications to the water control. In terms of the specific grasses, MICROBOOST reduced shoot growth of *Gliocladium* and *Bacillus* B69 treatments on fescue, *Trichoderma* kd and *Gliocladium* treatments on ryegrass and *Trichoderma* kd treatments on bentgrass (Table 5.11). Although not accepted at the 5% confidence level, the F value at 69 DAP was lower than that noted for 9-50 DAP. This suggests that MICROBOOST had more of an effect on microbial activity once the microbial population and the grass were established. Comparing (R^1) for Turf 2, reduced growth with MICROBOOST was noted only for *Trichoderma* kd and *Bacillus* B69 treatments on fescue (Table 5.14). Reduced growth as per the grass types was non-significant ($P \geq 0.05$).

Root length response to MICROBOOST applications to the treatments for specific grass types showed significant ($P \leq 0.05$) differences in length for Turf 2. Root lengths of treatments, with and without MICROBOOST, differed from the water controls, as well as between the microbial-based treatments at germination 9 DAP ($LSD_{(0.05)} = 3.428$) and 20 DAP ($LSD_{(0.05)} = 3.773$) (Table 5.14). On established grass, significant ($P \leq 0.05$) differences for MICROBOOST applications to the grass types (i.e., a mean over all MICROBOOST treatments and those without, for ryegrass, fescue and bentgrass) were noted 41-59 DAP for Turf 1 only (Table 5.13). This significant difference extended only to differences between ryegrass treatments with MICROBOOST and no MICROBOOST applications. At 69 DAP the effect of MICROBOOST on establishment was non-significant (Table 5.13 and 5.14). However, grass root length differences for treatments with MICROBOOST and without (R^1), showed that ryegrass was associated with reduced lengths for all applications of MICROBOOST to treatments (Table 5.13) . In Turf 2, this was only observed for *Trichoderma* kd and *Gliocladium* treatments (Table 5.14).

Reduced root growth was associated with *Gliocladium* treatments on bentgrass in Turf 1(Table 5.13).

A mean of treatments with and without MICROBOOST for each grass type is also presented in the tables. The mean of treatments with MICROBOOST showed a significant ($P \leq 0.05$) increase in shoot length for grasses in Turf 2 only. However, increased shoot lengths of ryegrass and bentgrass over both Turf 1 and 2 were observed. For roots there was no significant ($P \geq 0.05$) difference in lengths between treatments with MICROBOOST and those without. However, the mean for treatments with MICROBOOST showed increased root length for bentgrass in Turf 1 and 2. Fescue root lengths, Turf 1, were greater with MICROBOOST treatments but there appeared to be no growth stimulation in Turf 2. Growth stimulation of ryegrass also was inconsistent between the two trials. Figures 5.19 and 5.20 show the effect of a treatment means over the three grass types and Turf 1 and 2, for shoot and root lengths. From these figures, MICROBOOST applications had little effect on growth stimulation, as the differences were hardly noticeable.

Table 5.11 Table of means and ANOVAs of representative core shoot lengths (mm) for treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation trial Turf 1, conducted at Cedara (Feb- April, 2001)

		SHOOT LENGTHS (MM) MEASURED																								
		RYEGRASS									FESCUE									BENTGRASS								
		R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Treatments	Water	1	1	26.7	44.2	67.8	82.8	96.9	109	136	2	1	24.8	38.3	63.1	79.3	95.6	110	119	1	1	8.6	15.8	19.1	27.6	35.7	40.9	48.3
	Water + MICROBOOST	2		46.9	61.8	76.6	90.1	101	117	137	1		29.5	43.4	50.8	67.4	81.5	93.5	109	2		8.8	14.0	24.7	34.3	38.3	44.8	49.2
	Trichoderma kd	7	4	43.0	63.2	86.6	101	119	151	213	6	4	28.7	47.1	63.1	87.0	98.9	117	137	7	3	10.0	17.0	27.1	38.4	49.8	59.8	63.0
	Trichoderma kd + MICROBOOST	6		41.2	60.3	76.7	97.3	124	162	212	7		32.6	51.3	73.4	82.6	97.6	117	135	3		10.4	16.3	22.7	31.7	45.8	52.0	59.1
	Gliocladium	8	3	43.9	64.9	85.3	102	126	156	205	8	3	28.5	44.5	67.6	81.8	104	132	151	4	4	11.1	16.3	21.1	30.6	45.4	52.2	61.6
	Gliocladium + MICROBOOST	3		38.4	51.7	74.5	90.3	121	148	209	4		29.3	41.8	62.6	83.5	90.2	108	130	8		11.5	20.1	29.2	39.6	45.7	58.9	63.3
	Bacillus B69	4	2	34.0	57.0	81.3	96.3	110	153	210	5	2	28.2	51.6	59.4	76.0	93.5	113	131	5	2	11.8	17.2	25.7	35.1	41.2	49.6	62.5
	Bacillus B69 + MICROBOOST	5		42.5	58.2	77.3	102	121	159	202	3		26.8	44.2	65.6	78.6	94.9	104	128	6		11.9	17.4	28.1	33.5	43.2	53.8	63.0
	Mean of Trtms with MICROBOOST	2		42.3	58.0	76.3	94.9	117	146	190	1		29.6	45.2	63.1	78.0	91.1	106	125	2		11.0	17.0	26.2	34.8	43.2	52.4	58.7
	Mean of Trtm with NO MICROBOOST	1		36.9	57.3	80.3	95.8	113	142	191	2		27.6	45.4	63.3	81.0	98.0	118	134	1		10.0	16.6	23.3	32.9	43.0	50.6	58.8

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.11 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
LSD (0.05)	Microbes	6.03	6.92	7.96	6.99	8.29	6.87	5.57
	Grass.Microbes	10.44	11.99	13.78	12.11	14.36	11.9	9.65
	Grass.MICROBOOST	7.38	8.48	9.74	8.57	10.15	8.41	6.82
F probability (P)	Microbes	0.654 (NS)	0.308 (NS)	0.210 (NS)	0.05 *	0.006 **	< 0.001 ***	< 0.001 ***
	MICROBOOST	0.230 (NS)	0.906 (NS)	0.880 (NS)	0.780 (NS)	0.719 (NS)	0.357 (NS)	0.099 (NS)
	Grass.Microbes	0.989 (NS)	0.946 (NS)	0.977 (NS)	0.828 (NS)	0.703 (NS)	0.002 **	< 0.001 ***
	Grass.MICROBOOST	0.600 (NS)	0.988 (NS)	0.591 (NS)	0.712 (NS)	0.316 (NS)	0.017 **	0.148 (NS)
	Microbes.MICROBOOST	0.396 (NS)	0.402 (NS)	0.948 (NS)	0.726 (NS)	0.574 (NS)	0.472 (NS)	0.963 (NS)
	Grass.Microbes.MICROBOOST	0.533 (NS)	0.440 (NS)	0.357 (NS)	0.391 (NS)	0.930 (NS)	0.347 (NS)	0.322 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		7.14	8.2	9.42	8.28	9.81	8.13	6.6
CV%		27.2	20.5	17	11.9	11.7	7.9	5.2

DAP = days after planting

Table 5.12 Table of means and ANOVAs of representative core shoot lengths (mm) for treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation of trial Turf 2, conducted at Cedara (March - May, 2001)

Treatments	SHOOT LENGTHS (MM) MEASURED																									
	RYEGRASS									FESCUE									BENTGRASS							
	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Water	**1**	**1**	26.2	35.4	53.3	66.2	92.1	118	153	**1**	**1**	24.4	36.8	48.8	65.2	74.3	92.0	123	**1**	**1**	7.4	12.2	16.6	22.8	32.9	39.1	46.6
Water + MICROBOOST	**2**		27.6	41.2	58.7	79.2	101	126	166	**2**		25.9	38.0	52.5	66.7	79.9	99.2	130	**2**		8.8	14.5	20.5	26.7	33.5	44.9	51.4
Trichoderma kd	**5**	**4**	33.3	49.5	66.2	90.1	120	156	219	**8**	**4**	28.0	42.8	61.3	78.2	90.8	126	164	**5**	**3**	9.6	14.5	22.8	31.9	42.7	50.6	61.3
Trichoderma kd + MICROBOOST	**8**		32.4	48.3	71.2	91.6	128	169	233	**6**		29.7	41.2	59.5	74.6	92.5	118	157	**8**		9.9	16.6	24.3	31.8	43.6	53.5	63.4
Gliocladium	**3**	**2**	29.1	44.2	69.4	84.9	114	155	214	**4**	**3**	28.9	42.7	54.5	67.1	87.2	120	160	**4**	**2**	9.1	14.5	20.7	29.5	39.6	50.6	62.3
Gliocladium + MICROBOOST	**6**		30.1	46.4	66.9	89.8	123	166	217	**7**		28.4	42.8	63.8	73.4	90.3	124	155	**6**		10.3	14.6	20.3	32.5	41.9	52.5	61.5
Bacillus B69	**4**	**3**	31.2	46.3	68.6	86.7	121	155	215	**5**	**2**	29.5	42.7	56.2	72.1	88.9	119	156	**3**	**2**	9.5	15.1	22.4	28.9	38.0	48.2	57.1
Bacillus B69 + MICROBOOST	**7**		35.9	48.4	71.3	86.3	120	161	222	**3**		28.4	39.5	57.1	72.8	86.7	116	159	**7**		10.1	15.5	23.1	31.8	40.8	53.4	62.1
Mean of Trtms with MICROBOOST	**2**		31.5	46.1	67.0	86.7	118	156	209	**2**		28.1	40.3	58.2	71.8	87.4	114	150	**2**		15.3	22.0	30.7	39.9	51.0	59.6	32.6
Mean of Trtm with NO MICROBOOST	**1**		29.9	43.8	64.3	81.9	112	146	200	**1**		27.7	41.2	55.2	70.6	85.3	114	151	**1**		14.1	20.6	28.3	38.3	47.1	56.8	30.6

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.12 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
$LSD_{(0.05)}$	Microbes	1.044	1.69	2.304	3.03	4.271	6.78	5.076
	MICROBOOST	0.738	1.195	1.629	2.143	3.02	4.79	3.591
	Grass.Microbes	1.809	2.928	3.99	5.249	7.398	11.74 0	8.797
F probability (P)	Microbes	< 0.001 *** [a]	< 0.001 ***	< 0.001 ***	< 0.001 *** [a]	< 0.001 ***	< 0.001 ***	< 0.001 ***
	MICROBOOST	0.014 **	0.163 (NS)	0.005 **	0.011 **	0.035 *	0.065 (NS)	0.036 *
	Grass.Microbes	0.001 ***	0.002 **	0.005 **	0.047 *	0.017 *	0.013 **	< 0.001 ***
	Grass.MICROBOOST	0.426 (NS)	0.102 (NS)	0.710 (NS)	0.395 (NS)	0.416 (NS)	0.275 (NS)	0.104 (NS)
	Microbes.MICROBOOST	0.649 (NS)	0.173 (NS)	0.568 (NS)	0.097 (NS)	0.639 (NS)	0.904 (NS)	0.368 (NS)
	Grass.Microbes.MICROBOOST	0.066 (NS)	0.668 (NS)	0.066 (NS)	0.520 (NS)	0.938 (NS)	0.930 (NS)	0.862 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		1.813	2.935	4	5.262	7.416	11.77	8.819
CV%		8	8.8	8.4	8.5	9.3	11.2	6.4

DAP = days after planting

[a] = significant($P \leq 0.05$) deviations on interactions noted

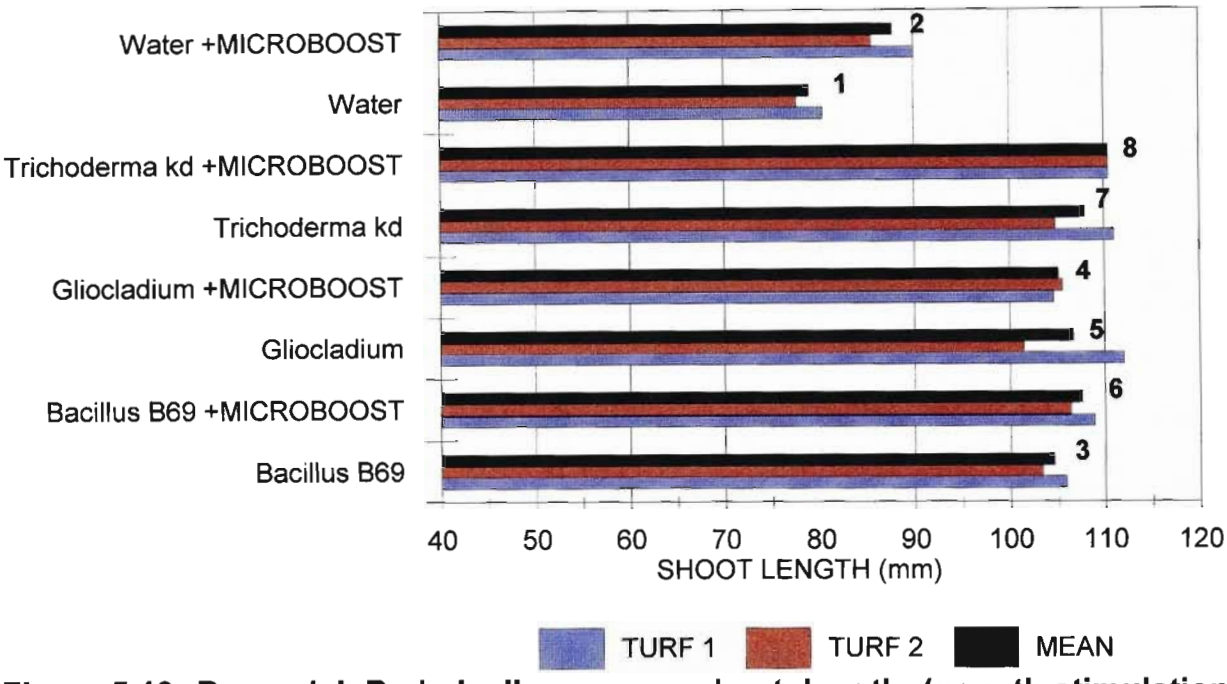


Figure 5.13 Perennial Prelude II ryegrass shoot length (growth stimulation) response to treatments for Turf 1 and 2, and a mean over both *in vivo* turf trials, conducted at Cedara (2001).

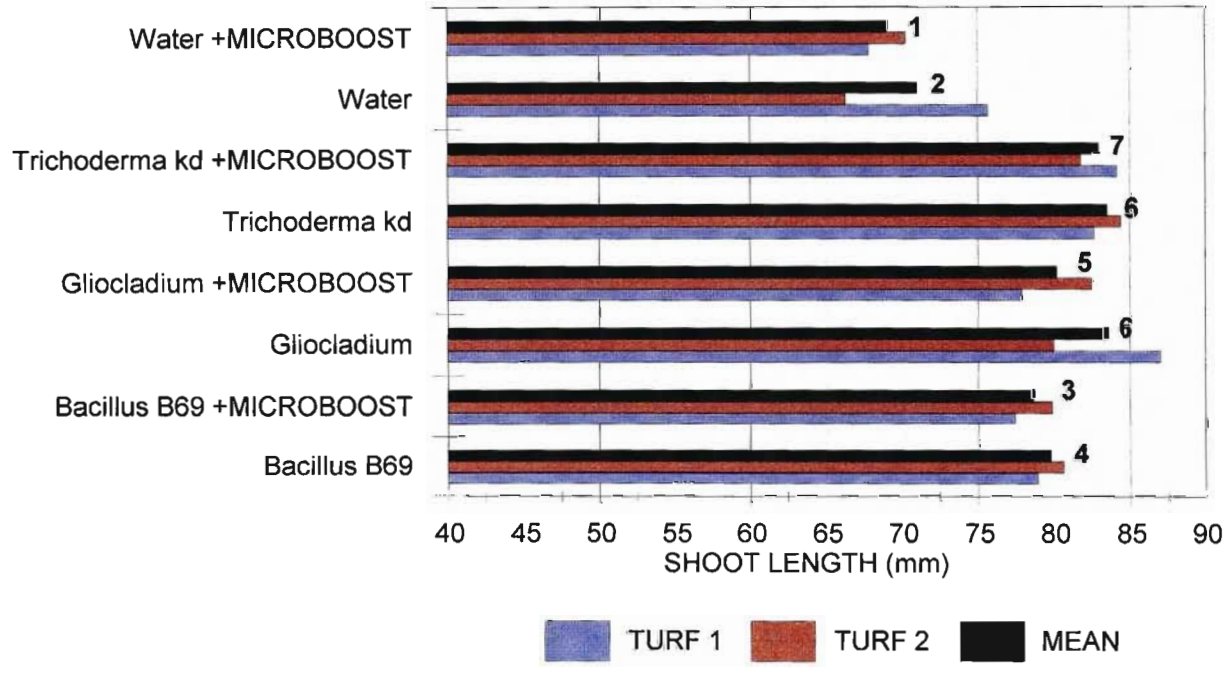


Figure 5.14 Junior fescue shoot length (growth stimulation) response to treatments for Turf 1 and 2, and a mean over both *in vivo* turf trials, conducted at Cedara (2001).

1-8 = ranking of the mean of treatment showing the effect of MICROBOOST

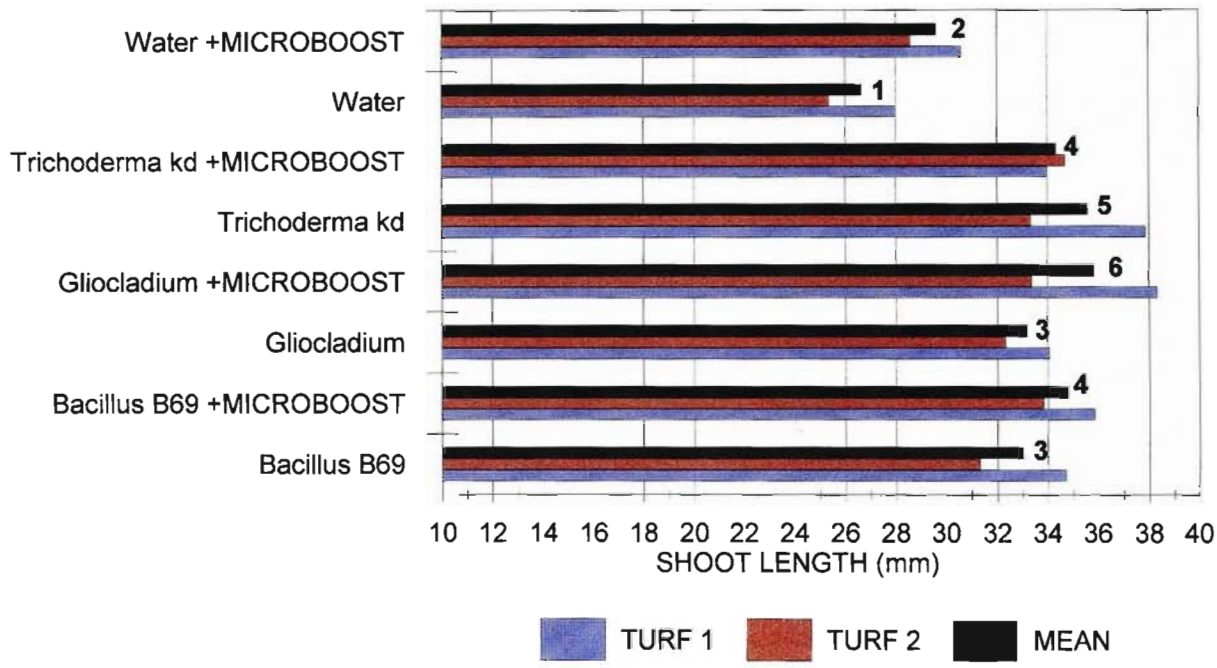


Figure 5.15 Crenshaw bentgrass shoot length (growth stimulation) response to treatments for Turf 1 and 2, and a mean over both *in vivo* turf trials, conducted at Cedara (2001).

1-6 = ranking of the mean of treatment showing the effect of MICROBOOST

Table 5.13 Table of means and ANOVAs of representative core root lengths (mm) for treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation trial Turf 1, conducted at Cedara (Feb - April, 2001)

		ROOT LENGTH (MM) MEASURED																									
		RYEGRASS									FESCUE									BENTGRASS							
		R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Treatments	Water	**2**	**1**	23.7	35.2	51.5	66.6	73.6	82.9	93.4	**1**	**1**	25.7	38.8	49.9	63.4	73.8	82.9	88.6	**1**	**1**	9.9	14.5	20.3	32.1	38.7	50.9	57.8
	Water + MICROBOOST	**1**		31.9	41.7	46.7	56.5	69.3	77.7	95.1	**2**		27.8	45.6	51.6	66.4	78.6	84.4	90.1	**6**		9.9	16.7	26.7	36.3	46.3	54	60.8
	Trichoderma kd	**7**	**3**	34.5	47.4	54.7	65.8	80	90.7	107	**6**	**4**	32.2	48.4	55.3	69.5	83	92.3	95.3	**3**	**2**	10.6	17	22.7	30.4	40.6	51.3	56.5
	Trichoderma kd + MICROBOOST	**4**		30.2	45.2	52.7	66.9	76.6	87.2	103	**8**		32.6	51.3	60.9	74.8	85.8	91.3	96.1	**7**		12.9	22.6	28.3	37.1	46.2	49	59.7
	Gliocladium	**8**	**4**	33.5	48.1	59	74	83.3	95.3	109	**3**	**2**	29.3	40.3	50.3	64.5	83.8	91.1	96.1	**8**	**3**	12.3	20.3	30.9	43	49.7	55.8	64.6
	Gliocladium + MICROBOOST	**6**		34.1	43.8	58	67.4	75	87.5	111	**4**		28.4	41.6	55.2	68.5	81.9	88.4	92.2	**5**		10.6	18	24.7	33	45.8	52.8	59.4
	Bacillus B69	**5**	**2**	28.7	43.5	54.1	67.7	75.9	91.1	102	**5**	**3**	31.5	48.1	57.1	65.5	77.5	84.9	92.3	**2**	**1**	13.2	16.8	23.5	29.6	37.7	44.7	61.7
	Bacillus B69 + MICROBOOST	**3**		28.7	45.1	54.1	65.5	70.4	80.6	110	**7**		27.9	42	56.1	75.8	85.6	94.3	98.3	**4**		12	18.3	24.8	30.4	41.5	50	64.8
	Mean of Trtms with MICROBOOST	**1**		31.2	43.9	52.9	64.1	72.8	83.3	105	**2**		29.2	45.1	55.9	71.4	82.9	89.6	94.2	**2**		11.4	18.9	26.1	34.2	44.9	51.5	61.2
	Mean of Trtm with NO MICROBOOST	**2**		30.1	43.6	54.8	68.5	78.2	90	103	**1**		29.7	43.9	53.2	65.7	79.5	87.8	93.1	**1**		11.5	17.2	24.4	33.8	41.7	50.7	60.2

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.13 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
$LSD_{(0.05)}$	Microbes	2.663	3.496	3.466	3.841	3.795	2.452	4.292
	Grass.Microbes	4.612	6.055	6.003	6.652	6.573	4.247	7.434
	Grass.MICROBOOST	3.261	4.281	4.244	4.704	4.648	3.003	5.256
F probability (P)	Microbes	0.027 *	0.008 **	0.021 *	0.075 (NS)	0.004 *	< 0.001 *** [a]	0.004 **
	MICROBOOST	0.863 (NS)	0.356 (NS)	0.468 (NS)	0.684 (NS)	0.732 (NS)	0.110 (NS)	0.358 (NS)
	Grass.Microbes	0.347 (NS)	0.317 (NS)	0.202 (NS)	0.079 (NS)	0.495 (NS)	0.002 **	0.182 (NS)
	Grass.MICROBOOST	0.748 (NS)	0.898 (NS)	0.248 (NS)	0.016 *	0.015 **	< 0.001 ***	0.956 (NS)
	Microbes.MICROBOOST	0.239 (NS)	0.183 (NS)	0.697 (NS)	0.117 (NS)	0.179 (NS)	0.101 (NS)	0.277 (NS)
	Grass.Microbes.MICROBOOST	0.417 (NS)	0.489 (NS)	0.366 (NS)	0.419 (NS)	0.939 (NS)	0.097 (NS)	0.860 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		3.153	4.139	4.104	4.548	4.494	2.903	5.082
CV%		13.2	11.7	9.2	8.1	6.7	3.8	5.9

DAP = days after planting

[a] = significant($P \leq 0.05$) deviations on interactions noted

Table 5.14 Table of means and ANOVAs of representative core root lengths (mm) for treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation trial Turf 2, conducted at Cedara (March - May, 2001)

Treatments		ROOT LENGTH (MM) MEASURED																								
		RYEGRASS									FESCUE									BENTGRASS								
		R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
	Water	1	1	26.2	30.8	34.4	46.1	53.6	61.6	70.4	1	1	23.5	27.0	35.0	48.5	56.0	64.4	70.7	1	1	12.3	15.7	18.4	24.8	53.6	37.3	42.7
	Water + MICROBOOST	2		25.6	31.6	36.8	49.6	56.7	64.6	72.4	2		26.8	29.7	34.9	46.6	53.6	63.5	70.9	2		13.5	16.8	20.5	27.5	56.7	42.5	48.3
	Trichoderma kd	7	4	31.3	38.8	47.4	57.4	67.1	79.2	98.9	3	2	29.6	26.9	46.0	57.5	70.1	80.3	90.3	3	2	13.6	16.9	20.4	27.7	67.1	47.2	54.7
	Trichoderma kd + MICROBOOST	6		29.3	35.6	44.8	57.5	67.7	80.6	97.7	6		28.7	36.1	45.4	59.0	69.1	79.2	90.0	7		16.2	18.8	22.5	28.2	67.7	45.8	57.5
	Gliocladium	5	2	27.1	34.6	43.6	57.8	69.8	78.3	96.9	7	3	29.2	35.6	45.6	58.2	68.2	79.9	91.9	6	3	15.2	18.7	21.8	27.1	69.8	47.6	55.6
	Gliocladium + MICROBOOST	4		26.8	34.6	43.9	56.9	68.1	75.6	97.4	8		32.2	39.9	47.1	59.4	69.7	80.5	92.4	8		13.9	18.6	22.9	31.3	68.1	49.7	56.3
	Bacillus B69	3	3	27.9	34.2	41.2	57.4	69.2	75.5	97.2	4	2	31.5	37.2	44.6	56.3	66.2	77.2	90.4	4	2	13.4	18.1	22.1	30.1	69.2	47.4	54.6
	Bacillus B69 + MICROBOOST	8		32.5	39.7	47.4	58.9	69.9	78.7	95.2	5		30.6	36.7	44.2	57.2	67.2	78.9	89.5	5		15.0	19.2	22.6	29.3	69.9	45.1	54.6
	Mean of Trtms with MICROBOOST	2		28.5	35.4	43.2	55.7	65.8	74.8	90.7	1		29.6	35.6	42.9	55.5	64.9	75.5	85.7	2		14.6	18.3	22.1	29.1	38.1	45.8	54.2
	Mean of Trtm with NO MICROBOOST	1		28.1	34.6	41.6	54.6	64.9	73.6	90.8	1		28.4	34.2	42.8	55.1	65.1	75.5	85.7	1		13.6	17.4	20.7	27.4	36.3	44.8	51.9

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.14 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
$LSD_{(0.05)}$	Microbes	1.399	1.54	2.033	2.149	2.04	2.219	2.059
	Grass.Microbes	2.424	2.668	3.521	3.722	3.534	3.844	3.567
	Grass.Microbes.MICROBOOST	3.428	3.773	4.979	5.263	4.998	5.436	5.044
F probability (P)	Microbes	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***	< 0.001 ***
	MICROBOOST	0.087 (NS)	0.059 (NS)	0.154 (NS)	0.172 (NS)	0.309 (NS)	0.354 (NS)	0.370 (NS)
	Grass.Microbes	0.019 * [a]	0.002 **	0.006 **	0.013 **	< 0.001 ***	0.005 **	< 0.001 ***
	Grass.MICROBOOST	0.798 (NS)	0.895 (NS)	0.633 (NS)	0.813 (NS)	0.506 (NS)	0.819 (NS)	0.294 (NS)
	Microbes.MICROBOOST	0.549 (NS)	0.291 (NS)	0.671 (NS)	0.956 (NS)	0.697 (NS)	0.590 (NS)	0.379 (NS)
	Grass.Microbes.MICROBOOST	0.032 * [a]	0.054 * [a]	0.449 (NS)	0.611 (NS)	0.345 (NS)	0.373 (NS)	0.950 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		2.43	2.675	3.53	3.731	3.543	3.854	3.576
CV%		10.2	9.2	9.9	8.1	6.3	5.9	4.7

DAP = days after planting

[a] = significant($P \leq 0.05$) deviations on interactions noted

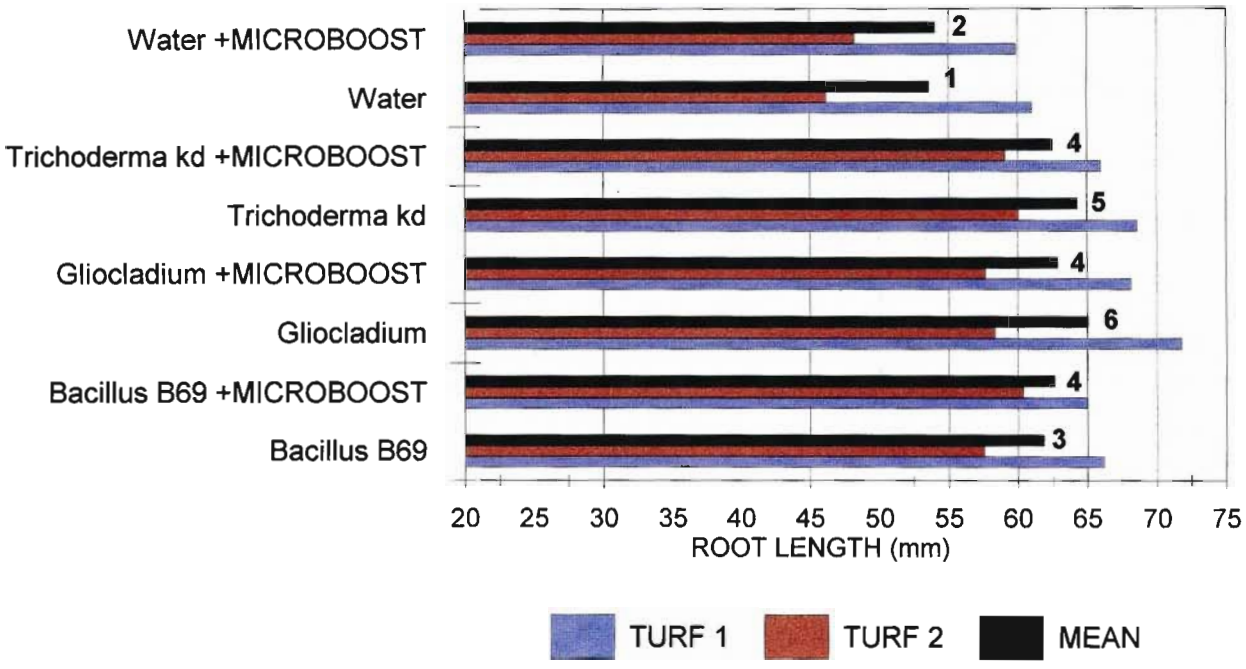


Figure 5.16 Perennial Prelude II ryegrass root length (growth stimulation) response to treatments for Turf 1 and 2, and a mean over both *in vivo* turf trials, conducted at Cedara (2001).

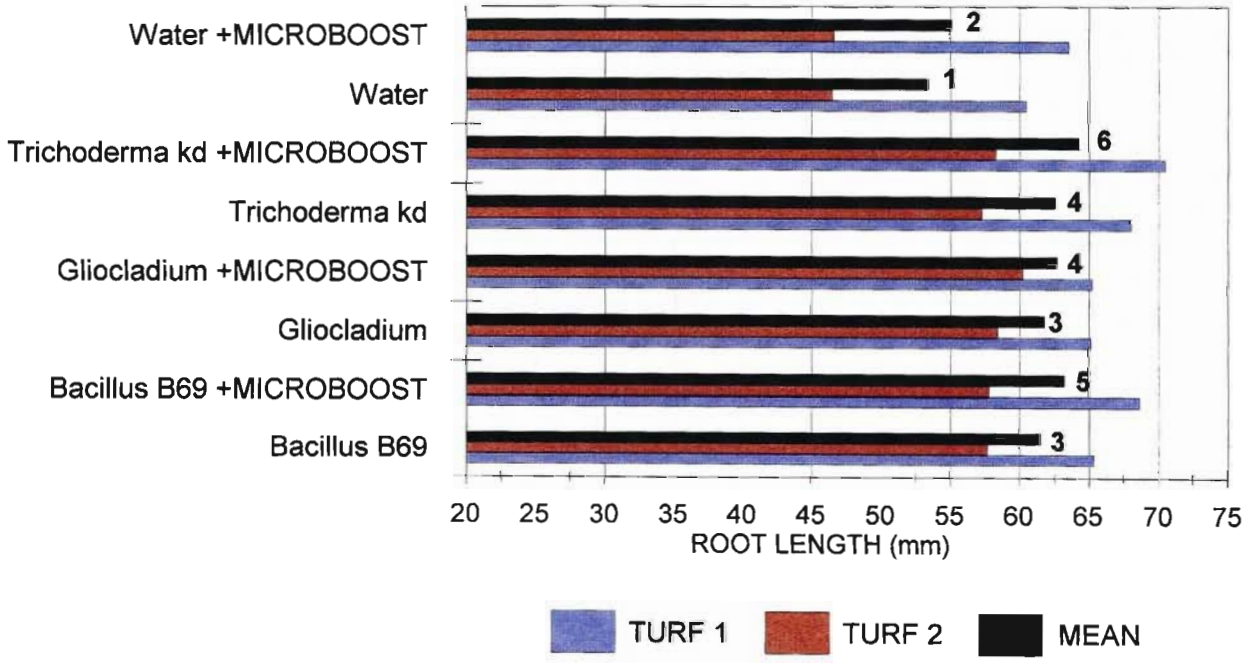


Figure 5.17 Junior fescue root length (growth stimulation) response to treatments for Turf 1 and 2, and a mean over both i*n vivo* turf trials, conducted at Cedara (2001).

1-6 = ranking of the mean of treatment showing the effect of MICROBOOST

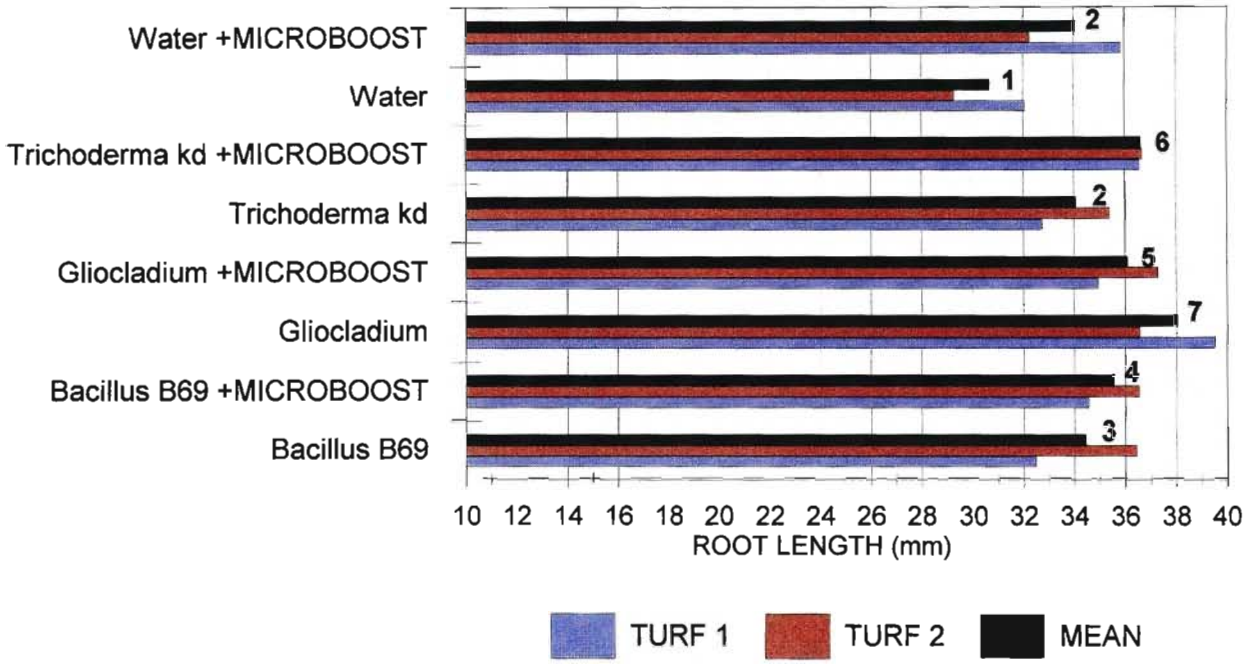


Figure 5.18 Crenshaw bentgrass root length (growth stimulation) response to treatments for Turf 1 and 2, and a mean over both *in vivo* turf trials, conducted at Cedara (2001).

1-7 = ranking of the mean of treatment showing the effect of MICROBOOST

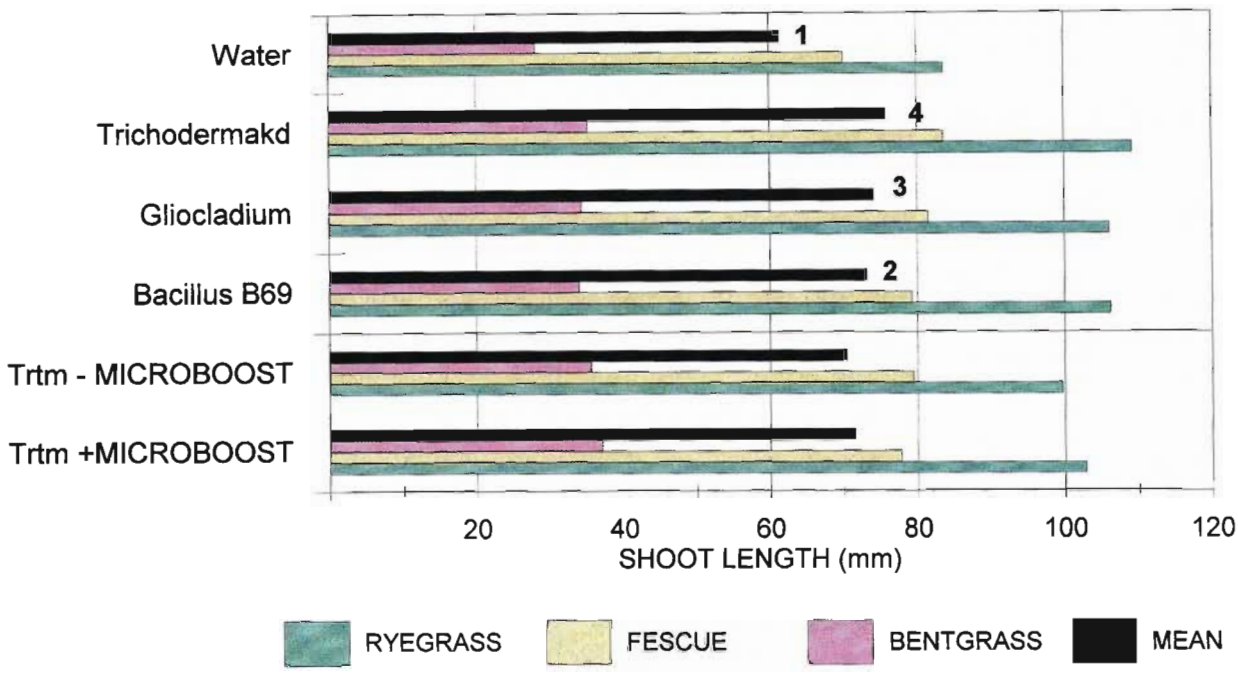


Figure 5.19 Mean (over Turf 1 and 2) shoot length response to the microbial-based treatments versus the water control. *In vivo* turf trials, conducted at Cedara (2001).

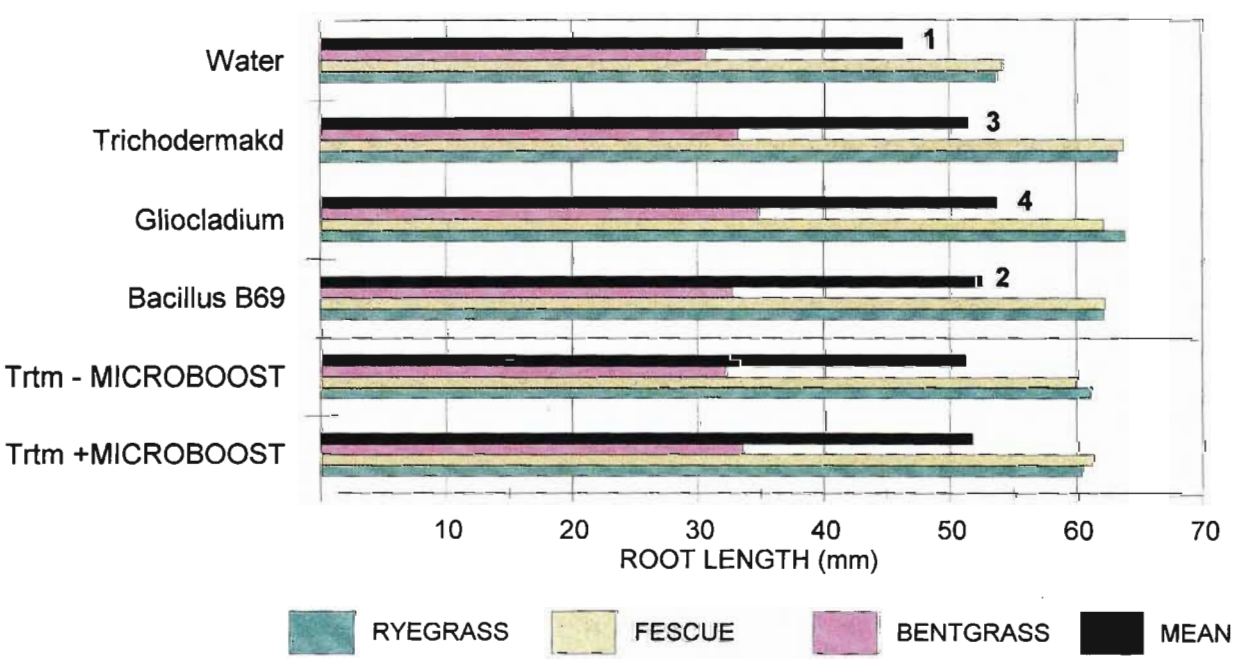


Figure 5.20 Mean (over Turf 1 and 2) root length response to microbial-based treatments versus the water control. *In vivo* turf trials, conducted at Cedara (2001).

1-4 = ranking of the mean of control-based and microbial-based treatments

Weed growth

Tables 5.15 and 5.16 show diagonal weed counts for ryegrass, fescue and bentgrass plots, Turf 1 and 2. ANOVA is also summarised in Tables 5.15 and 5.16. Significant ($P \leq 0.05$) weed growth for grass types and microbe treatments (i.e., *Trichoderma* kd, *Gliocladium* and *Bacillus* B69) versus the water control were shown. Only one significant difference was shown in Turf 1. For Turf 2, the water-based treatments were significantly associated with the least number of weeds encountered (Table 5.16). A significant ($P \leq 0.05$) increase in weed growth was also caused by MICROBOOST applications to the treatments. Significant associations ($P \leq 0.05$) were, however, concluded to be once off as they were inconsistent. The CV%'s were also very high.

To identify treatment trends, weed counts over the trial period were ranked for the effect of microbial treatments (R^1) and the effect of microbe- versus water-based treatments (R^2). Weed responses to microbial treatments for grass types were inconsistent. Inconsistent weed growth was also observed between Turf 1 and 2. For example, water-based treatments accounted for the least number of weeds encountered for all grass types in Turf 2 (Table 5.16), but only for fescue in Turf 1 (Table 5.15). MICROBOOST applications to treatments also caused variable weed growth. A mean of the treatment effects with and without MICROBOOST over Turf 1 and 2 showed increased weed growth associated with MICROBOOST applications for ryegrass only (Figure 5.21).

Figure 5.22 presents a mean of weed growth for microbe- versus the water-based treatments of all three grass types for Turf 1 and 2. *Gliocladium*-based treatments accounted for the least number of weeds observed (Rank 1), with water-based treatments accounting for the second lowest. This was observed for plant counts in Figure 5.12 as well. *Trichoderma* kd and *Bacillus* B69 caused the greatest weed incidence over the trial periods. All microbe-based treatments initially (50 DAP) showed higher weed growth than the control. From 50 DAP, weed growth for the microbe-based treatments was reduced with control plots showing the highest numbers of weeds (69 DAP). At 40 DAP, all weed numbers appeared to drop due to increased establishment of grasses comprising the treatment plots.

Table 5.15 Table of means and ANOVAs (square root transformed data) of representative diagonal weed counts for treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation trial Turf 1, conducted at Cedara (Feb - April, 2001)

		NUMBER OF WEEDS ENCOUNTERED																									
		RYEGRASS									FESCUE									BENTGRASS							
		R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R^1	R^2	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Treatments	Water	7	3	1.5	7.3	11.0	8.5	7.5	6.0	5.5	5	1	1.3	6.3	8.0	6.5	7.0	5.5	5.5	6	4	4.0	3.8	6.0	7.0	8.0	6.5	5.5
	Water + MICROBOOST	4		3.3	2.3	8.5	6.5	5.5	6.0	5.5	4		1.5	2.0	5.5	6.5	8.0	7.0	5.0	7		2.8	2.5	6.5	9.5	9.5	7.5	7.0
	Trichoderma kd	3	2	4.3	4.5	6.5	5.5	5.5	4.0	2.0	2	4	4.3	2.5	8.5	5.5	3.5	3.0	5.0	8	3	1.8	5.5	10.5	10.0	10.0	8.0	5.5
	Trichoderma kd + MICROBOOST	2		1.5	2.8	5.5	6.0	7.5	5.5	3.0	8		2.3	3.8	8.5	13.0	11.0	8.0	8.0	2		2.8	2.8	6.5	6.0	5.5	5.0	4.0
	Gliocladium	1	1	1.5	2.3	7.0	3.5	2.5	2.5	3.0	7	3	2.8	4.5	8.0	10.5	12.0	9.0	5.5	3	1	3.0	3.5	7.5	7.0	7.5	5.0	3.0
	Gliocladium + MICROBOOST	5		2.8	2.5	8.0	8.0	8.5	6.5	3.0	1		2.8	2.8	4.5	5.5	5.5	4.0	3.5	1		2.3	2.0	8.0	6.5	4.5	4.5	3.5
	Bacillus B69	6	4	1.5	7.0	10.0	8.5	8.0	5.5	3.5	6	2	3.3	2.8	5.0	9.0	9.0	8.0	5.5	5	2	2.8	2.0	5.0	6.5	9.0	7.0	6.0
	Bacillus B69 + MICROBOOST	8		2.5	2.8	10.0	11.5	10.5	11.0	7.5	3		3.3	4.8	7.0	6.0	5.0	4.5	3.5	4		1.3	3.0	7.0	8.0	7.5	6.0	4.5
	Mean of Trtms with MICROBOOST	2		2.5	2.6	8.0	8.0	8.0	7.3	4.8	2		2.4	3.3	6.4	7.8	7.4	5.9	5.0	2		2.3	2.6	7.0	7.5	6.8	5.8	4.8
	Mean of Trtm with NO MICROBOOST	1		2.2	5.3	8.6	6.5	5.9	4.5	3.5	1		2.9	4.0	7.4	7.9	7.9	6.4	5.4	1		2.9	3.7	7.3	7.6	8.6	6.6	5.0

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.15 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
LSD $_{(0.05)}$	Grass.Microbes.MICROBOOST	3.553	4.316	5.176	8.331	6.537	5.795	4.571
F probability (P)	Microbes	0.901 (NS)	0.685 (NS)	0.926 (NS)	0.736 (NS)	0.518 (NS)	0.358 (NS)	0.142 (NS)
	MICROBOOST	0.992 (NS)	0.195 (NS)	0.337 (NS)	0.800 (NS)	0.993 (NS)	0.504 (NS)	0.898 (NS)
	Grass.Microbes	0.375 (NS)	0.833 (NS)	0.111 (NS)	0.723 (NS)	0.649 (NS)	0.759 (NS)	0.449 (NS)
	Grass.MICROBOOST	0.758 (NS)	0.512 (NS)	0.808 (NS)	0.726 (NS)	0.164 (NS)	0.130 (NS)	0.473 (NS)
	Microbes.MICROBOOST	0.918 (NS)	0.473 (NS)	0.934 (NS)	0.988 (NS)	0.807 (NS)	0.995 (NS)	0.889 (NS)
	Grass.Microbes.MICROBOOST	0.290 (NS)	0.824 (NS)	0.690 (NS)	0.212 (NS)	0.028 *	0.101 (NS)	0.478 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		0.82	0.92	0.45	0.78	0.63	0.59	0.52
CV%**		59.4	55.9	16.8	29.5	24	25	24.9

DAP = days after planting

Table 5.16 Table of means and ANOVAs (square root transformed data) of representative diagonal weed counts for treatments applied to perennial ryegrass, fescue and bentgrass in a field growth stimulation trial Turf 2, conducted at Cedara (March - May, 2001)

		NUMBER OF WEEDS ENCOUNTERED																								
		RYEGRASS									FESCUE									BENTGRASS								
		R[1]	R[2]	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R[1]	R[2]	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69	R[1]	R[2]	DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
Treatments	Water	1	1	0.0	0.0	0.0	1.0	1.0	1.0	1.0	2	1	0.0	1.0	1.5	2.5	2.5	3.0	1.5	1	1	0.5	2.0	2.0	1.5	0.5	0.5	1.5
	Water + MICROBOOST	2		0.0	2.0	0.5	0.0	1.0	1.5	2.0	6		0.0	5.0	6.0	4.5	2.5	2.0	3.0	3		0.0	1.5	3.0	3.0	0.5	2.0	2.5
	Trichoderma kd	4	4	1.0	0.5	1.5	2.0	2.0	1.5	1.5	7	1	2.5	3.0	4.0	4.5	3.5	3.5	3.5	6	2	1.0	0.5	5.0	4.5	0.5	2.0	2.0
	Trichoderma kd + MICROBOOST	8		1.0	4.0	5.0	4.5	2.0	2.0	1.5	1		0.0	0.5	2.5	2.5	2.0	1.5	1.5	2		0.5	2.0	1.5	2.5	1.0	1.5	2.0
	Gliocladium	3	3	0.0	1.0	1.0	2.5	1.5	1.0	1.5	5	2	1.5	2.5	4.0	4.5	2.5	2.0	2.0	8	4	0.5	4.5	4.0	3.5	2.5	3.5	3.5
	Gliocladium + MICROBOOST	7		0.0	3.0	3.0	4.0	1.0	2.0	3.0	4		0.5	1.5	4.0	4.0	3.0	3.0	2.0	7		1.5	2.0	3.0	2.5	2.5	3.0	2.5
	Bacillus B69	5	2	1.5	2.0	2.5	2.0	1.5	1.0	0.5	7	3	0.5	3.5	5.0	5.0	4.5	3.5	2.5	4	3	0.5	2.0	2.0	3.0	2.0	2.0	2.5
	Bacillus B69 + MICROBOOST	6		1.0	3.0	2.5	1.5	1.0	2.0	1.5	3		0.5	2.5	2.5	2.5	2.0	3.0	1.5	5		0.5	3.0	2.5	2.5	2.0	2.5	1.5
	Mean of Trtms with MICROBOOST	2		0.5	3.0	2.8	2.5	1.3	1.9	2.0	1		0.3	2.4	3.8	3.4	2.4	2.4	2.0	1		0.6	2.1	2.5	2.6	1.0	2.3	2.1
	Mean of Trtm with NO MICROBOOST	1		0.6	0.9	1.3	1.9	1.5	1.1	1.1	2		1.1	2.5	3.6	4.1	3.3	3.0	2.4	2		0.6	2.3	3.3	3.1	1.4	2.0	2.4

DAP = days after planting

R^1 = ranking between treatments for each grass type over the trial period

R^2 = ranking of a mean for control-based treatments in comparison to a mean for each of the microbe-based treatments

Table 5.16 cont....

		DAP 9	DAP 20	DAP 31	DAP 41	DAP 50	DAP 59	DAP 69
	Microbes	0.838	1.288	1.078	1.174	1.314	1.034	0.959
	Grass.Microbes	1.452	2.23	1.867	2.034	2.276	1.791	1.661
	Grass.MICROBOOST	1.027	1.577	1.32	1.438	1.609	1.266	1.175
	Grass.Microbes.MICROBOOST	2.054	3.154	2.641	2.877	3.218	2.533	2.349
	Microbes	0.115 (NS)	0.244 (NS)	0.025 *	0.011 **	0.686 (NS)	0.348 (NS)	0.535 (NS)
	MICROBOOST	0.361 (NS)	0.106 (NS)	0.251 (NS)	0.708 (NS)	0.464 (NS)	0.522 (NS)	0.869 (NS)
	Grass.Microbes	0.611 (NS)	0.762 (NS)	0.03 *	0.513 (NS)	0.790 (NS)	0.411 (NS)	0.873 (NS)
	Grass.MICROBOOST	0.526 (NS)	0.035 *	0.041 *	0.968 (NS)	0.944 (NS)	0.324 (NS)	0.302 (NS)
	Microbes.MICROBOOST	0.642 (NS)	0.262 (NS)	0.096 (NS)	0.499 (NS)	0.671 (NS)	0.691 (NS)	0.443 (NS)
	Grass.Microbes.MICROBOOST	0.806 (NS)	0.094 (NS)	0.111 (NS)	0.053	0.832 (NS)	0.878 (NS)	0.765 (NS)
Degrees of freedom (residual)		23						
Standard error (s.e.) Grass.Microbes.MICROBOOST		0.6424	0.6374	0.4464	0.5177	0.698	0.5094	0.5491
CV%**		137.4	49.1	28.6	32.5	60.1	37.4	41.6

DAP = days after planting

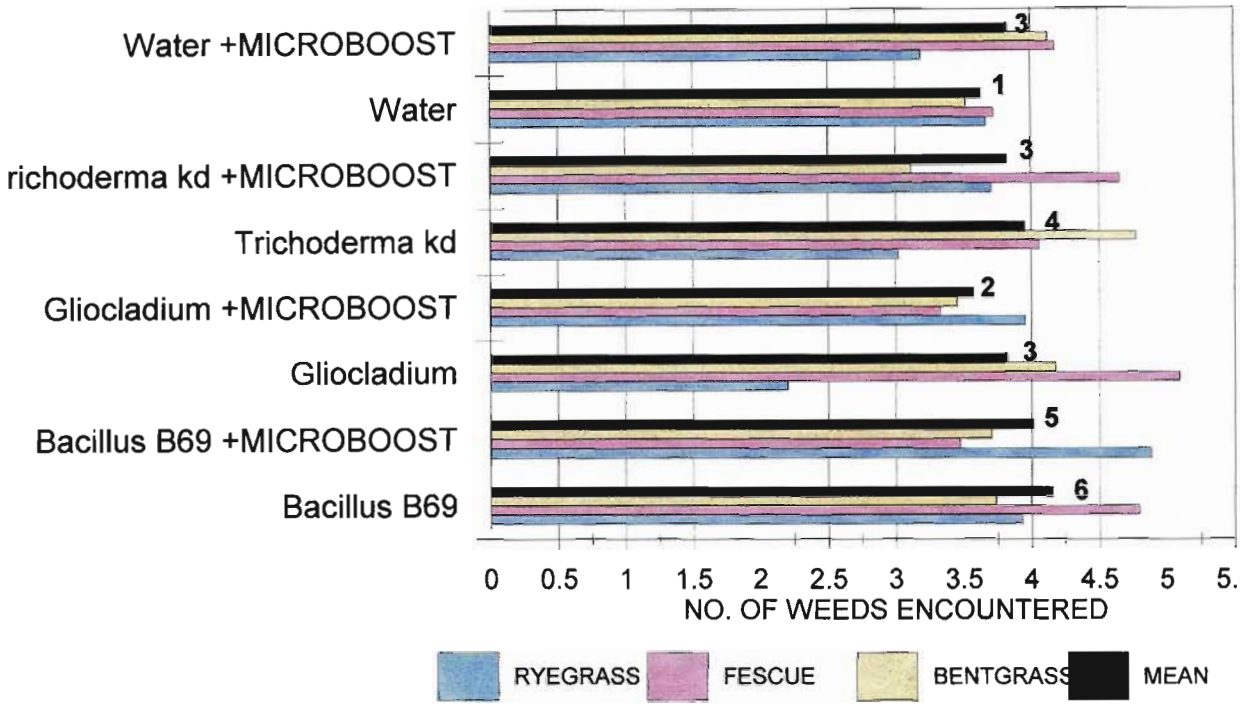


Figure 5.21 Effect of treatments in terms stimulated weed growth for grass types over Turf 1 and 2 *in vivo* turf trials, conducted at Cedara (2001).

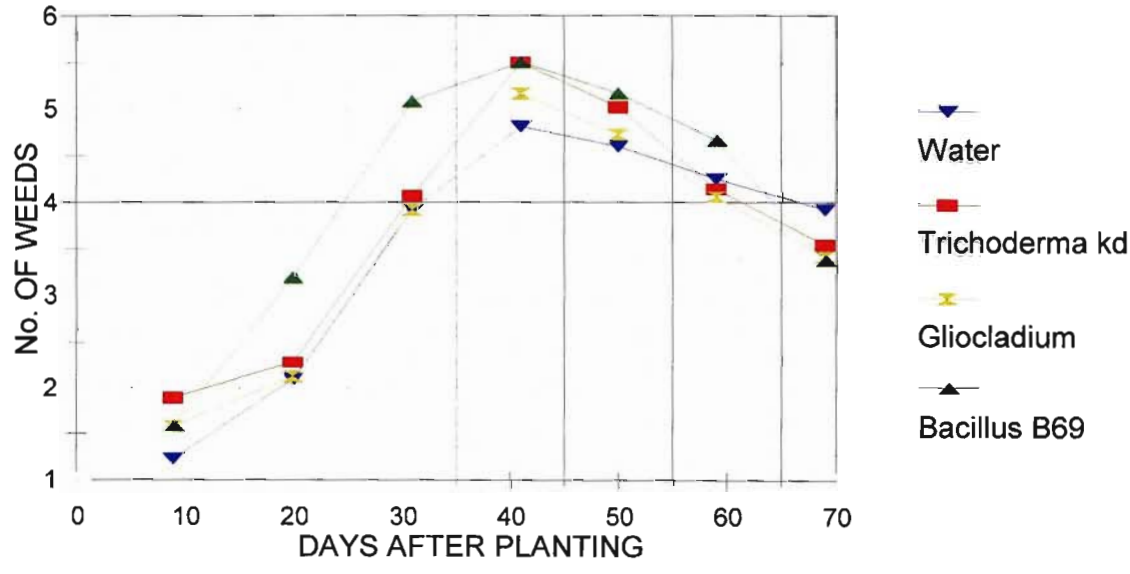


Figure 5.22 Effect of microbe-based treatments versus water-based control for the trial period, over grass types for Turf 1 and 2 *in vivo* turf trials, conducted at Cedara (2001).

5.4 DISCUSSION

Biological control agents have the potential to increase plant growth (Baker, 1992; Kapulnik, 1996; Raviv *et al.,* 1998; Harman, 2000). However, being living organisms, their activity is determined by the environment in which they occur (Papavizas, 1985; Andersch, 1992; Koch, 1999). Cook (1990) found that *Bacillus* spp. applied to seeds of cereal crops, including oats (*Avena sativa* L.) and wheat (*Triticum aestivum* L.) caused increased seedling emergence and establishment, in both a controlled greenhouse environment and a field trial. High variability did, however, exist between the trials. This is similar to what was observed for these growth stimulation trials, where high variability in the field trials for the same treatments on the same grass type, caused different growth trends. Data manipulation was applied and revealed potential growth stimulation trends for treatments.

The aim of these *in vitro* and *in vivo* trials was to determine growth stimulation with microbial-based treatments. *In vivo* testing was determined during autumn/winter when, in the Midlands Mistbelt area (Bioresource group 5), rainfall is low, night temperatures may fall below freezing and severe frosts can occur (Camp, 1995).Temperate grasses were chosen for establishment of the trials, with perennial and annual ryegrass used for the *in vitro* pot trials. Temperate grasses have the potential to establish well under warmer temperatures, if moisture is sufficient (Schroeder and Sprague, 1996). Moisture was adequate for both *in vitro* and *in vivo* trials. A computerized datalogger, situated at Cedara's weather station, recorded daily minimum and maximum temperatures, rainfall, relative humidity and leaf wetness (Anon, 2001). For the *in vitro* trial it was assumed that conditions within the greenhouse were maintained at an optimum for plant growth.

In vitro *observations*

The *in vitro* pot trial was not repeated. For a more sound approach, repetition is required. From this trial, perennial ryegrass showed increased germination rates with *Trichoderma* kd (Table 5.3). From the compatibility tests in chapter 4, *T. harzianum* was more aggressive than *B. subtilis*. *Trichoderma* kd should therefore, establish itself quicker in the growing medium, colonizing the rhizosphere on seed germination.

Significant differences were noted between the microbe-based treatments and water control for initial (i.e., 6 DAP) germination % and shoot length of perennial and annual ryegrass. These were, however, inconsistency between the two grasses. Increased growth caused by the application of *Trichoderma* kd could be due to the increased availability of plant nutrients, especially nitrogen (Harman, 2000). Increased nitrogen availability generally results in increased growth. Both perennial and annual ryegrass have high fertility and moisture requirements (Bartholomew, 1991). In terms of shoot length and increased DW, *Bacillus* B69 accounted for the greater increment. *Bacillus* sp. are also known to improve nutrient uptake through enhanced root development (Burr and Caesar, 1984). Of the microbial treatments, *Bacillus* B69 treatments caused increased growth and ranked highest for most observations. Plant growth inhibition with *Trichoderma* sp. applications have been noted (Ousley *et al.*, 1993). However, in comparison to the control treatment *Trichoderma* kd ranked higher, thus growth inhibition was not shown here.

In consistency between perennial and annual ryegrass can also be attributed to the seed, in that no batch of seed will have 100% germination rate. The number of seeds used for trial establishment (30 per treatment) was probably also too low for meaningful results to be attributed to the treatments applied. Planting depth is another factor that will affect germination % and establishment rates. This should be considered for further studies.

In vivo *observations*

Established under field conditions, grass plants and microbes were exposed to a large number of growth variables that would have caused the great variation in growth stimulation results observed. *In vivo* testing is, however, vital to determine what would happen under commercial field production situations. Treatment growth responses observed *in vivo* were not only inconsistent between the two trials, but high variability was also found within trials. Due to the trial design, treatments applied and treatment application intervals being identical for both trials, a mean over both trials was determined. The mean reduced the variability in growth stimulation results between Turf 1 and 2, compensating for environmental influences. Due to the growth stimulation fluctuations between trials, treatment means of Turf 1 and 2 were invalid. The treatment means did, however, show potential trends but these would require further investigation.

Germination and establishment rates

Survival of amended microbes is vital for plant responses to be noted. Both *Bacillus* and *Trichoderma* spp. form resting spores for survival (Papavizas, 1985; Weller, 1988). A threshold microbial population in the soil must also be maintained for consistent results (Weller, 1988). This is achieved by means of frequent inoculations. Manipulation of the soil environment to ensure optimum conditions for survival will also encourage antagonistic populations (Nigam and Mukerji, 1988). This is, however, achieved easier *in vitro* than *in vivo.* To increase microbial activity, the microbial activator MICROBOOST was applied with microbial treatments, MICROBOOST supplying a readily available nutrient source. According to Krebs *et al.* (1998) nutrition is a major factor effecting antagonistic activity of *Bacillus* spp. and phytopathogenic fungi.

Temperature *in vitro* was maintained at approximately 23-25^{0}C for the duration of the trial. *In vivo,* daily air temperature variations were greater for Turf 1, where Turf 1 experienced temperatures ranging from 7-35^{0}C, where as for Turf 2 the range was from 4-27^{0}C. However, it is variation in soil temperature, away from an optimum, that will have the greatest influence on microbial populations (Tate, 1995). Soil temperature was,

however, not determined for these trials. Soil temperature requirement for the establishment of temperate grasses is only 5^0C (Carpenter *et al.*, 1990). Optimum *B. subtilis* (*Bacillus* B69) activity has been tested at temperatures of 17-25^0C, although some activity is noted at 10^0C (Krebs *et al.*, 1998). *Trichoderma harzianum* colonization is optimal at temperatures in the range of 20-30^0C (Kleifeld and Chet, 1992; Ousley *et al.*, 1994). Low soil temperatures will therefore have played a large role in reducing the antagonist's population size colonizing the rhizosphere, and having no effect on plant growth stimulation.

Endosperm nutrient reserves are sufficient for seed germination to occur. However, upon emergence of the radical and plumule, minerals are absorbed from the growing medium for further growth. *In vitro* a significant increase in seedling emergence (6 DAP) was caused by the microbial treatments (Tables 5.3 and 5.4). Increased germination with microbial treatments could be attributed to the production of plant growth regulators (Kleifeld and Chet, 1992; Harman, 2000). Increased microbial activity caused by MICROBOOST applications also resulted in a significant increase in root DW of annual ryegrass, where DW of roots treated with *Trichoderma* kd + MICROBOOST differed significantly ($LSD_{(0.05)}$ = 11.6) from those of the control treatment (Table 5.8). For spore germination, *T. harzianum* conidia require a nutrient source (Danielson and Davey, 1972). In this trial MICROBOOST may have supplied this external nutrient source, resulting in increased conidial germination, rhizosphere colonization and plant growth stimulation. Although non-significant ($P \geq 0.05$), increased DW of perennial ryegrass (Table 5.7) could also be associated with the increase in seed germination associated with MICROBOOST applications (Table 5.3). Increased seed germination resulted in better plant establishment.

Shoot lengths associated with treatments + MICROBOOST were increased for perennial ryegrass. However, the control + MICROBOOST treatment also caused increased shoot length in comparison to the water control (Table 5.5). This was also noted for increased shoot and root DW of annual and perennial ryegrass(Table 5.7 and 5.8), with greater DW

accounting for greater growth. MICROBOOST supplies a readily available nutrient source for improved microbial activity. Growth stimulation associated with the control + MICROBOOST treatment suggests that increased nutrition may have also caused increased plant growth stimulation. However, this is unlikely as MICROBOOST is specifically formulated for increasing microbial activity. Indigenous antagonists may have been present in the soil, MICROBOOST applications increasing their activity and thus growth stimulation observed. Indigenous microbes were not confirmed before the amendment of treatments. Growth stimulation associated with the control + MICROBOOST treatment does suggest that instead of applying "processed" antagonistic microbes it would be easier to stimulate indigenous antagonistic populations already established in the soil (Papavizas, 1985). *In vivo* MICROBOOST had no significant ($P \geq 0.05$) effect on increased microbial activity. MICROBOOST applications had variable effects on microbial activity and seed germination (Tables 5.9 and 5.10) and overall decreased microbial activity was noted. Due to the high variability and high CV%'s no conclusions were made. A mean for MICROBOOST was determined over all treatments (as shown in the means tables for germination counts, shoot and root lengths and weed growth) in an attempt to compensate for variability in order to observe treatment trends.

Of the grass types, ryegrass showed growth stimulation for all treatments with MICROBOOST. Of the grasses established, perennial ryegrass is the one most reliant on well fertilized soils (Schroeder and Sprague, 1996). The increased growth response of ryegrass may thus be due to an increased nutrient availability associated with increased microbial activity (Burr and Caesar, 1984; Harman, 2000). In terms of increased microbial activity associated with MICROBOOST applications, conclusions were drawn from the means presented in Figure 5.12. Rankings of the treatments showed *Trichoderma* kd + MICROBOOST caused greater seed germination than *Trichoderma* kd alone. The differences were, however, small. Increased *T. harzianum* activity with MICROBOOST applications was observed for all grass types (comparing rankings of Figures 5.7, 5.8 and 5.9).

The "delay" in significant differences noted for Turf 1 in terms of microbe effects on germination (Table 5.9) in comparison to Turf 2 (Table 5.10) could therefore be due to the heavy downfall of rain experienced. For Turf 1, the microbial treatments caused a significant increase in grass establishment and coverage at 69 DAP (Table 5.9). For Turf 2 significant differences occurred, but increased germination caused by the microbial treatments in comparison to the control treatment was non-significant ($LSD_{(0.05)} = 17.97$). Germination differences between *Gliocladium* and other microbial treatments was, however, significant. *Gliocladium* treatments resulted in the lowest establishment population, while *Trichoderma* kd treatments resulted in the highest rate (Figure 5.11). Classification and characteristics of *Trichoderma* and *Gliocladium* spp. are very similar in their teleomorph and biology (Papavizas, 1985; Samuels, 1996). Both fungi are also considered as antagonistic soilborne mycoparasites, that are aggressive colonizers of the rhizosphere (Handelsman and Stabb, 1996; Yu and Sutton, 1999; Harman, 2001). It has been hypothesised that application of microbes would simply act to enhance microbial populations that are already present naturally in the soil. Reduced activity of *Gliocladium* in comparison to other microbes could be based on the assumption that *Gliocladium* populations in the soil were absent or very low, or that the strain and formulation used was not particularly active.

However, plant growth stimulation was assumed to be associated with rhizosphere colonization of the amended antagonists upon seed germination. This would offer suppression of soilborne pathogens and improve water and nutrient uptake of the plant roots (Burr and Caesar, 1984; Kapulnick, 1996; Harman, 2000). However, *in vivo* there are a number of factors to consider in terms of successful root colonization. It has also been suggested that *T. harzianum* is not associated with increased nutrient uptake by plants (Inbar *et al.*, 1994).

To reduce inconsistency in results between Turf 1 and 2, fertilizer application timing and amount applied was the same for both trials. Temperature, however, has a notable effect on fertilizer use, with nitrogen responses increasing with increasing temperature

(Whitehead, 1970). For example, Crenshaw bentgrass will tolerate high temperatures during summer when combined with a high nitrogen fertilization program (White and Smithberg, 1980). However, for ryegrass and fescue, high nitrogen levels are not conducive to a good turf establishment, as high temperature and high nitrogen levels lead to a lack of persistence and the development of tufts which are easily pulled from the soil (Tainton *et al.,* 1976). Temperature differed for the two trials with Turf 2 experiencing cooler temperatures.

Moisture availability is another important consideration affecting seed germination and microbial activity (Weller, 1988). Treatments were reapplied as a drench for both *in vitro* and *in vivo* trials. It is speculated that water percolating through the growing medium will carry amended microbes to the root tips, where colonization will occur (Bahme and Schroth, 1987). Within the greenhouse a 6hr irrigation cycle existed, emitting 30-50mm water/week. It was noted that pots at the back of the benches received less water than those at the front (confirmed by the presence of algal growth in the front pots). This could have contributed to the variable growth *in vitro* and the reason for the high CV% (>20%) for annual ryegrass. Annual ryegrass was placed slightly off-center from the sprinkler nozzle in comparison to the perennial ryegrass pots, creating an edge effect. This would also account for variability. However, the randomised block design and replicates should have reduced this variability.

In vivo plots received 25mℓ water per week on a 72hr cycle. However, variable results for Turf 1 could be accounted for by a heavy downfall of rain experienced 4 DAP and after the initial treatment application. Many of the seeds had not yet germinated and were vulnerable to wash. As a result of this rainfall, two replicates were removed from the trial due to excessive wash, leaving only two replications for data sampling. This would account for the greater variability observed in Turf 1. A further reason could be that, grass seed requires only a light raking followed by rolling at planting in order to provide adequate seed coverage (Tainton and Klug, 2002). The impact of the raindrops, associated with the downpour soon after planting (Turf 1), would have physically

disturbed the soil particles resulting in the seeds being covered by too much soil. High variability between Turf 1 and 2 was noted for plant counts for bentgrass (Figures 5.10 and 5.11), where Turf 1 showed much lower establishment. Bentgrass seeds measure only 2mm in length, while ryegrass and fescue seeds display bigger spiklets with seeds measuring from 10mm in length. Being pushed deeper into the soil by the force of the raindrops, the final depth would have been too great for emergence to occur. Decreased germination could also have been due to the formation of a soil crust (Vengris and Torello, 1982), as noted once the trial block had dried out. Due to the finer texture of the bentgrass seeds, soil crusting will have impacted greatest on bentgrass emergence.

Water movement through the soil profile will also impact on microbial distribution in the soil (Weller, 1988). It was previously stated that water percolating through the growing medium will carry amended microbes to the root tips (Bahme and Schroth, 1987). However, due to the heavy downfall the initial application of treatments in Turf 1 could have been washed from the seeds beyond the rhizosphere. Growth stimulation could be due to the control of soilborne diseases (Harman, 2000). Poor colonization of the rhizosphere by antagonists would account for the high variability in treatment rankings (Table 5.9), where the water control treatment often showed a greater establishment rate (i.e. final grass coverage/population of plants) than the microbe-based treatments. This was, however, more noticeable in Turf 2. Reduced germination in Turf 2, was therefore thought to be associated primarily with the lower temperatures and thus microbial activity within the soil. To confirm this, microbial activity would have to be determined by means of observing activity/colonization when exposed to different temperature regimes.

It is important to consider the validity of determining a treatment mean over three different grass types due to differences in growth habits. Both perennial ryegrass and fescue are tufted grasses, perennial ryegrass leaf blades measuring up to 4mm in width and fescue up to 10mm. Bentgrass has a creeping growth habit, has leaf blades measuring 2mm in width and thus has a much finer texture (Van Oudtshoorn, 1992). From the trial plots, bentgrass was much harder to count due to its fine texture. Plot wash in Turf 1 disturbed

the even seed distribution, with seeds being washed into "clumps" creating uneven patches of germination, which added to the difficultly of counting (Figure 5.26). Of the three grasses, fescue took longest to germinate, with ryegrass emerging before bentgrass. Different growth patterns would account for variability in germination (Figures 5.7-5.9 and Tables 5.9 and 5.10). Based on the materials and methods carried out, variability between Turf 1 and 2 should have been minimal, but as observed in the figures, it was not. This could largely be due to the heavy rainfall experienced at planting of Turf 1. This would hold true in that significant differences ($P \leq 0.05$) for establishment were noted for bentgrass in Turf 2 (Table 5.10). Significant differences in germination for the grass types also existed in Turf 1, where *Bacillus* B69 accounted for greater germination of bentgrass than the control. This was, however, only significant at germination (9 and 20 DAP) (Table 5.9). Significance noted for bentgrass is questionable, in that where increased germination was expected with *Bacillus* B69, ryegrass displayed a significant decrease in germination.

Establishment was determined as plant counts. However, this was difficult due to the very fine leaf blades at emergence and it was also time consuming due to the large number of treatment plots. Although still using a personal judgement of percent soil coverage, the compilation of a rating scale using digital pictures from which coverage has been determined based on colour (i.e. grass and soil) would prove advantageous. Assessment of the whole plot instead of a sample area, would also be achieved using this method.

Figure 5.26 Uneven soil coverage observed for Turf 1, bentgrass with Bacillus B69 treatment (white residue of talcum powder noticeable). Upper left hand corner shows poor/no turf emergence, while the lower right hand corner shows better establishment (Cedara, 2001).

Shoot and root growth

For both Turf 1 and 2, microbial treatments increased both root and shoot growth (Tables 5.11-5.14, Figures 5.13-5.18). The differences in root and shoot growth were significant, with increased shoot lengths associated with the microbial treatments in Turf 2 (Table 5.12) being highly significant from emergence to establishment. Significant increases in shoot lengths for Turf 1 (Table 5.11) were, however, not present at 9 DAP. This may have been due to delayed rhizosphere colonization by the antagonists caused by the heavy downfall experienced at planting, also resulting in reduced seed germination and emergence associated with the microbial treatments. However, increased root lengths associated with the treatments (Tables 5.13 and 5.14) were significant from emergence to establishment.

An extensive root system would increase nutrient and moisture uptake, resulting in increased shoot growth. Increased root and shoot growth (Figures 5.19 and 5.20) could have been caused by increased plant nutrient availability due to increased microbial activity on the rhizosphere associated with the application of MICROBOOST. Differences, however, were too small to consider. Significant differences ($P \leq 0.05$) were noted for shoot length (Table 5.12) for treatments with MICROBOOST and for root lengths for treatments with MICROBOOST but only for specific grass types (Table 5.13). Increased root and shoot growth was again noted for the water + MICROBOOST treatment (Tables 5.11-5.14 and Figures 5.13, 5.15-5.18). The effect of MICROBOOST would best be determined by means of *in vitro* testing where increased microbial population would be determined by means of microbial counts, determined both in the absence and presence of a host plant.

Increased root lengths may not have been a true reflection of growth stimulation associated with the microbial treatments. The growth habit of the grasses (Van Oudtshoorn, 1992) would have influenced root and shoot lengths. For example, growth stimulation of bentgrass would have been better represented by determining top growth rather than measuring the very fine roots. However, leaf removal has shown to stop root growth entirely due to the plant entering into shock, reducing plant growth (Tainton and Klug, 2002). Roots and shoots were obtained from cores removed from the treatment plots. Core removal should provide some evidence of root activity with approximately 85% of turfgrass roots being found in the top 120mm of soil (Tainton and Klug, 2002). However, coring is a destructive means of sampling and thus only one core was removed per treatment plot. Damage to roots at the cutting edge would have occurred, but root lengths recorded were, as far as possible, limited to the inside of the cores to reduce this affect. Cores also often contained only short new root growth which was also measured.

Increased root lengths observed would have been influenced by the presence of weed roots, because once shoots were removed it was impossible to determined grass from weed roots. Where possible the cores were removed from a weed-free area. Weeds

included in the cores were also removed. Comparing root lengths (Figure 5.20) and the number of weeds encountered (Figure 5.21 and 5.22) as a mean over the three grasses, it was evident that where weed growth was minimal, grass growth was optimal. *Gliocladium* treatments resulted in the greatest root length, with fewer weeds encountered in comparison. This effect was also evident for shoot growth (Table 5.21). In contrast, *Bacillus* B69 accounted for the highest number of weeds, and of the microbial treatments the lowest root and shoot lengths. Increased weed growth can also be associated with increased shoot lengths (not shown in these trials). Increased leaf area could result in shading of tillers (as for bentgrass) resulting in a patchy turf, which is prone to weed invasions (Tainton and Klug, 2002). Shoot lengths of bentgrass (Figures 5.13) for *Trichoderma* kd treatments, for example, ranked just lower than *Gliocladium* treatments but the number of weeds encountered was high (Figure 5.15).

Increased root and shoot lengths (Figures 5.19 and 5.20) were not associated with increased germination and soil coverage (Figure 5.12). *Bacillus* B69 treatments accounted for increased germination, but root and shoot measurements ranked lowest (ranks 2) of the microbial treatments (Figures 5.19 and 5.20). This was also shown to occur for the control treatment.

Where a low plant population exists, reduced inter-plant competition will occur potentially resulting in larger plants. Although a higher plant population would be perceived as more desirable, under suboptimal growth conditions increased competition between plants will result in self-thinning (Lush and Franz, 1991). Increased shoot growth, will result in plants that are able to absorb more sunlight, photosynthesising more sugars, producing stronger plants which are able to withstand extensive wear (Tainton and Klug, 2002). Such plants will produce an extensive root system, as shown in Figures 5.20. Disease resistance will also be increased upon colonization by the antagonist, provided optimum growth conditions occur.

Weed growth

Increased weed growth associated with the amended microbes was expected in that it was assumed that the microbes would have shown no host specificity, increasing weed germination and establishment as well. Significant differences between weed counts for the control versus microbial treatments were noted in Turf 2 on ryegrass (Table 5.16). However, at germination (9 DAP) and establishment (69 DAP), growth stimulation differences were non-significant ($P \geq 0.05$). ANOVA showed high CV%s even after data transformation (square root) and therefore significance noted was to be considered unlikely. The CV%s were, however, seen to decrease over the trial period indicating improved sample technique. Inconsistent weed counts were shown for treatment types and grass types.

Microbial treatments + MICROBOOST showed fewer weeds. Differences between treatments for number of weeds encountered was very small. This observation was determined as a mean over Turf 1 and 2 for all grass types which, as discussed for germination and emergence rates, is questionable. Trends observed from the mean would require further verification, using a single species of grass or, in the case of turfgrass production, a commercial mix. Significance noted for the effect of MICROBOOST on weed growth was too variable. For example, significant differences were shown for ryegrass Turf 2 at early establishment of the weeds (20 and 31 DAP, Table 5.16). Treatments without MICROBOOST accounted for fewer weeds, as was expected. This was, however, not true for other grass types. Non-significant differences in weed growth associated with treatments + MICROBOOST could have been due to weeds becoming obscured by the grass, increasing shoot growth was also associated with MICROBOOST applications (Table 5.12). Increased weed growth with microbial applications and MICROBOOST should have been determined by means of increased dry weight. Germination would have been stimulated, as determined by the weed counts, but actual survival and growth rate of these weeds was not determined.

Inconsistencies in weed numbers between the trials existed. Turf 1 showed increased weeds in comparison to Turf 2, due to the heavy rainfall experienced after planting resulting in a delay in grass seed germination. This delay in germination and emergence would have allowed weeds to become established first. Treatment means over the three grass types in Turf 1 (Figure 5.10) showed low grass germination to be associated with the water control. A mean for weed counts over the three grasses in Turf 1 (Table 5.15), showed the control to be associated with the second lowest number of weeds encountered. However, it was assumed that there was some relation between germination and weed growth in that, where *Gliocladium* caused increased grass germination weed numbers were decreased. For Turf 2 (which is considered to be the more accurate model of what would occur *in vivo*) germination and weed growth associated with *Gliocladium* were correlated, *Gliocladium* treatment causing increased weeds and reduced grass germination. Therefore, although weed growth was also stimulated by the microbes, the grass was more aggressive due to growth stimulation associated with rhizosphere colonization by the amended antagonists, thus outcompeting weeds. This is shown in Figure 5.22, where weed growth increased with grass growth but then decreased at 40 DAP once the grasses became established. This was shown for all treatments, including the control, which accounted for the least number of weeds at germination (9 DAP) but the most weeds encountered at trial termination (69 DAP).

Increased weed counts were also caused by plot wash, as ryegrass seedlings were often seen to germinate in bentgrass plots, thus the ryegrass was recorded as a weed. Another factor effecting weed presence would be the growth habit of the grass species observed. Of the grasses established, fescue displays an open sward, which excludes weeds (Shildrick 1980). However, for both Turf 1 and 2, fescue plots accounted for the greatest number of weeds. This could be due to fescue emerging later in comparison to other grasses.

Variability in weed counts could have also been due to the four weed types observed, some being more prominent than others. Uneven germination or weed seed distribution in the seedbank would also have resulted in inconsistent weed emergence within the treatment plots. Weed counts would have been better determined by means of individual weed identification recorded per treatment plot.

With or without microbial amendments, weed growth will occur requiring control measures to be implemented. Advances in herbicides allow for easy management of weeds in a grassland (Haggar, 1980, Tainton and Klug, 2002). Here, microbial applications for growth stimulation of the grasses, will increase initial weed growth so that the number of post-emergent herbicide applications required for total weed control will be reduced. Such a hypothesis would require further verification. Herbicides used for amenity purposes should have no negative effects on microbe populations.

Concluding remarks

Results generally showed growth stimulation trends with the amendment of antagonistic microbes, in comparison to the water control. Both *in vitro* and *in vivo* trials displayed much variation in trends observed for grass type and microbial amendments. Trends identified here would need further verification as to their validity. It can, however, be determined from the trials that microbial amendments increase germination, increasing the plant's aggressive establishment thus reducing weed competition and final weed population. The application of microbes did have a positive impact on growth stimulation, in terms of increased root and shoot growth, influencing percent cover at establishment and also the plant's ability to withstand severe wear and the ability to outcompete weeds.

5.5 REFERENCES

Agrios, G. 1997. Plant pathology, 4th edition. Academic Press, California: United States of America.

Andersch, W. 1992. Production of fungi as crop protection agents. In: Pflanzenschutz-Nachrichten Bayer 45/1992 (63). Bayer AG, Geschaftsbereich Pflanzenshutz, Leverkusen: Germany. p. 129-142.

Anon, 2001. Weather. http://agriculture.kzntl.gov.za/crop_protection/weather_records (accessed June 2001)

Anon, 2000. Genstat for Windows. Release 4.2, 5th edition. VSN International Ltd, Oxford: United Kingdom.

Askew, D. 1991. *Trichoderma* in the control of damping-off in containerized seedlings. MSc thesis, Department of Microbiology and Plant Pathology, University of Natal, Pietermaritzburg: South Africa.

Bahme, J.B. and Schroth, M.N. 1987. Spacial-temporal colonization patterns of a rhizobacterium on underground organs of potatoes. Phytopathology **77:** 1093-1100.

Baker, R. 1992. Biological control of diseases of crops grown in covered and environmentally controlled structures. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 231-241.

Bartholomew, P.E. 1991. Adaption of pastures species. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg; South Africa.

Burr, T.J. and A. Caeser. 1984. Beneficial plant bacteria. In: B.V. Conger (ed). Critical reviews in plant science, Volume 2:1. CRC Press, Florida: United States of America. p. 1-20.

Camp, K.G.T. 1995. The bioresource units of KwaZulu-Natal. Cedara Report N/A/95/32. Department of Agriculture and Environmental Affairs, Cedara, Pietermaritzburg: South Africa.

Carpenter, J.A, K.G. Boyce, D.G. Cameron, W.J. Collins, J.N. Read, B. Ross and A. Williams. 1990. Pastures. In: R.L. Reid (ed). The manual of Australian agriculture. Butterworths, Sydney: Australia. p. 228-280. Cited by: P.E. Bartholomew. 2000. Establishment of pastures in humid regions. In: N.M. Tainton (ed). 2000. Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 167.

Cook, R.J. 1990. Twenty-five years of progress towards biological control. In: D. Hornby (ed). Biological control of soil-borne plant pathogens. C.A.B International, Wallingford: United Kingdom. p. 1-14.

Danielson, R.M. and C.B. Davey. 1972. Effects of nutrients and acidity on phialospore germination of *Trichoderma in vitro.* Soil Biology and Biochemistry **5:** 517-524.

Fravel, D.R. 1992. Systems for efficient delivery of microbial biocontrol agents to soil. In: E.C. Tjomas, G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America. p. 399-413.

Haggar, R.J. 1980. Weed control and vegetation management with herbicides. In: I.H. Rorison and R. Hunt (eds). Amenity grassland, an ecological perspective. John Wiley & Sons, Salisbury: United Kingdom. p.163-173.

Handelsman, J. and E.V. Stabb. 1996. Biocontrol of soilborne plant pathogens. The Plant Cell **8:** 1855-1869.

Harman, G.E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease **84:** 377-393.

Harman. G.E. 2001. *Trichoderma* spp., including *T. harzianum, T. viride, T. koningii, T. hamatum* and other spp. Cornell Univeristy: Geneva. www.nysaes.cornell.edu/ent/biocontrol/pathogens/trichoderma.

Inbar, J., M. Abramsky, D. Cohen and I. Chet. 1994. Plant growth enhancement and disease control by *Trichoderma harzianum* in vegetable seedlings growth under commercial conditions. European Journal of Plant Pathology **100:** 337-346.

Kapulnik, Y. 1996. Plant growth promotion by rhizosphere bacteria. In: Y. Wisel, A. Eshel and U. Kafkafi (eds). Plant roots: the hidden half, 2nd edition. Marcel Dekker, New York: United States of America. p. 769-781.

Kleifeld, O. and I. Chet. 1992. *Trichoderma harzianum*: interaction with plants and effect on growth response. Plant and Soil **144:** 267-272.

Knudsen, G.R., D.J. Eschen, L.M. Dandurand and L. Bin. 1991. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum.* Plant Disease **75:** 466-470.

Koch, E. 1999. Evaluation of commercial products for microbial control of soil-borne plant diseases. Crop Protection **18:** 119-125.

Krebs, B., B. Höding, S. Kübart, M. Alemayehu Workie, H. Junge, G. Schmiedeknecht, R. Grosch, H. Boschow and M. Hevesi. 1998. Use of *Bacillus subtilis* as a biocontrol agent.I. Activities and characterization of *Bacillus subtilis* strains. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz **105:** 181-197.

Lo, C.T. 1998. General mechanisms of action of microbial biocontrol agents. Plant Pathology Bulletin **7:** 155-166.

MacVicar, C.N. 1991. Soil classification. A taxonomic system for South Africa. Memoirs on the Agricultural Resources of S.A. No. 15. Department of Agricultural Development, Pretoria: South Africa. p. 257.

Nigam, N. and K.G. Mukerji. 1988. Biological control - concepts and practices. In: K.G. Mukerji and K.L. Garg (eds). Biocontrol of plant diseases, Volume 1. CRC Press, Florida: United States of America. p. 1-14.

Ousley, M.A., J.M. Lynch and J.M. Whipps. 1993. Effect of *Trichoderma* on plant growth: a balance between inhibition and growth promotion. Microbial Ecology **26:** 277-285.

Ousley, M.A., J.M. Lynch and J.M. Whipps. 1994. The effects of addition of *Trichoderma* inocula on flowering and shoot growth of bedding plants. Scientia Horticulturae **59:** 147-155.

Papavizas, G.C. 1985. *Trichoderma* and *Gliocladium*: biology, ecology and potential for biocontrol. Annual Review of Phytopathology **23:** 23-54.

Raviv, M., B.Z. Zaidman and Y. Kapulnik. 1998. The use of compost as a peat substitute for organic vegetable transplant production. Compost Science and Utilization **6:** 46-52.

Samuels, G.J. 1996. *Trichoderma*: a review of biology and systematics of the genus. Mycological Research **100:** 923-935.

Schroeder, C.B. and H.B. Sprague. 1996. Turf management handbook, 5th edition. Interstate Publishers, Illinois; United States of America p. 5-112.

Schroth, M.N. and J.O. Becker. 1990. Concepts of ecological and physiological activities of rhizobacteria related to biological control and plant growth promotion. In: D. Hornby (ed). Biological control of soil-borne plant pathogens. CAB International, Wallingford: United Kingdom. p. 389-414.

Shildrick, J.P. 1980. Species and cultivar selection. In: I.H. Rorison and R. Hunt (eds). Amenity grassland, an ecological perspective. John Wiley & Sons, Salisbury: United Kingdom. p.69-99.

Tainton, N.M., D.I. Bransby and P. de V, Booysen. 1976. Common veld and pasture grasses of Natal. Shuter and Shooter, Pietermaritzburg: South Africa. p. 32-33.

Tainton, N.M. and J. Klug. 2002. The cricket pitch and its outfield. University of Natal Press, Pietermaritzburg: South Africa.

Tate,R.L. 1995. Soil microbiology. John Wiley & Sons, New York: United States of America. p. 181-185.

Van Oudtshoorn, F.P. 1992. Guide to grasses of Southern Africa. BRIZA Publikasies, Arcadia: South Africa. p. 82, 210,234.

Vengris, J. and W.A. Torello.1982. Lawns: basic factors, construction and maintenance of fine turf areas, 3rd edition. Thomson Publications, California: United States of America.

Weller, D.M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology **26:** 379-407.

White, D.B. and M.H. Smithberg.1980. Cold acclamation and deacclimation in cool-season grasses. In: J.B. Beard (ed). Proceedings of the 3rd international turfgrass research conference. American Society of Agronomy, Madison: United States of America. p. 149-154.

Whitehead,D.C. 1970. The role of nitrogen in grassland productivity. Bulletin 48. Commonwealth Agricultural Bureau, Farnham Royal; United Kingdom. Cited by: N. Miles and A.D. Manson. 2000. Nutrition of planted pastures. In: N.M. Tainton (ed). 2000. Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 187.

Windham, M.T., Y. Elad and R. Baker. 1986. A mechanism of increased growth induced by *Trichoderma* spp. Phytopathology **76:** 518-521.

Yu, H. and J.C. Sutton. 1999. Density dynamics of *Gliocladium roseum* in relation to biological control of *Botrytis cinerea* in red raspberry. Canadian Journal of Plant Pathology **21:** 23-32.

CHAPTER 6

DETERMINATION OF THE POTENTIAL FOR PREFERENTIAL GRAZING ON PASTURES TREATED WITH BIOCONTROL AGENTS FOR GROWTH STIMULATION

ABSTRACT

Concerns about an animal's health after grazing a pasture that has been inoculated with biocontrol agents were raised in a pasture production survey of the KwaZulu-Natal Midlands over 1999/2000. Grazing habits of Dohne Merino sheep, as a representative group of grazers, were observed to determine if any preference was given to pastures inoculated with biocontrol agents at Cedara ($29^0$32'S, $30^0$17'E). Formulations of the biocontrol agents *Bacillus subtilis* Ehrenberg & Cohn., *Trichoderma harzianum* Rifai and *Gliocladium virens* Miller, Gidens, Foster & von Arx, were applied to trial plots within a ryegrass pasture (*Lolium multiflorum* Lam.) from establishment and thereafter bi-weekly until trial termination six months later. Sheep displayed no grazing preference. In terms of growth stimulation, measured as increased wet and dry biomass, ryegrass treated with the biocontrol agent *T. harzianum,* showed greater wet and dry biomass (g) when compared to those treated with other microbes: *G. virens* and *B. subtilis*, and the water control. Dry matter percentage was calculated as a % of dry weight over wet weight. Here the water control showed the highest dry matter yields. This was non-significant ($P \geq 0.05$).

6.1 INTRODUCTION

In the pasture production survey 1999/2000 (Chapter 3), farmers indicated that they would consider using biological control agents (BCAs) if pasture palatability was not affected and the BCAs had no pathological effect on the grazers.

Public concern about the use of living microorganisms on food sources is a major disadvantage associated with biocontrol. However, there have been few recorded incidences of antagonistic microorganisms having direct pathological effects. Public concern is largely based on the potential rather than hard evidence (Curl and Truelove, 1986; Lynch, 1992). There should therefore, be more concern expressed when the withholding periods of agrochemicals are violated. In terms of palatability, the use of antagonistic rhizosphere microorganisms has been shown to enhance root and plant development through improved water and nutrient availability and uptake (Kapulnik, 1996; Harman, 2000).

Farmer's concerns are therefore, in general attributed to a lack of understanding of biocontrol. This is especially true in terms of an understanding about the microorganisms used, their mechanisms of action and the regulations that must be met before their registration and use. This chapter addresses the potential for growth stimulation or ability to withstand grazing stress associated with the use of BCAs. The grazing habits of grazers were also monitored to assess if there was any preference for grasses treated with BCAs.

6.2 MATERIALS AND METHODS

Trial site

This field trial was conducted at Cedara (Department of Agriculture and Environmental Affairs), situated in the KwaZulu-Natal Midlands, approximately 32km inland from

Pietermaritzburg. The soil was a well-drained, deep sandy-clay Hutton form (MacVicar,1991).

Trial design

The trial design (Figure 6.1) was that of a Latin square, with four treatments and four replicates, laid out into 16, $2m^2$ plots.

plot 1 **Water**	**plot 2** ***Bacillus***	**plot 3** ***Gliocladium***	**plot 4** ***Trichoderma***
plot 8 ***Trichoderma***	**plot 7** ***Gliocladium***	**plot 6** **Water**	**plot 5** ***Bacillus***
plot 9 ***Bacillus***	**plot 10** **Water**	**plot 11** ***Trichoderma***	**plot 12** ***Gliocladium***
plot 16 ***Gliocladium***	**plot 15** ***Trichoderma***	**plot 14** ***Bacillus***	**plot 13** **Water**

Figure 6.1 Latin square plot design of the grazing preference trial conducted at Cedara (April-September 2002).

Trial site preparation and establishment

Six weeks prior to the commencement of the trial, a representative soil sample (topsoil), as outlined by Miles (1991), was taken from each of the 16 small plots comprising the trial. A representative sample was submitted to the Soil Analysis and Fertilizer Advisory Service[1] for analysis, in terms of soil acidity and fertility status. The results showed soils with a permissible acid saturation of 6%, thus no liming was required.

[1] Soil Analysis and Fertilizer Advisory Service, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200. Tel: (+27)33 343371 ext 321

Phosphorus (P) in the form diammonium phosphate and at a rate of 1.3kg $64m^{-2}$ (200 kg P ha^{-1}), and nitrogen (N) at a rate of 18kg N ha^{-1}, were applied to the area two days before planting. No potassium (K) was required. A topdressing of N (Urea; NH_4 46%N) was applied at the first grazing, at a rate of 320g $64m^{-2}$ (50 kg ha^{-1}). Fertilizer application coincided with pre-emergent weed control, consisted of the establishment of a fine tilth achieved via conventional land preparation. This entailed first discing the area to a depth of 150mm.

Once the new weed flush was 50mm high (three weeks later), the area was disced again to a depth of 150mm, together with the application of fertilizers (as mentioned above). The area was then harrowed and rolled with a Cambridge roller in preparation for planting. No herbicide was applied.

Annual Exalta ryegrass was hand-planted at a seeding rate of 6g $2m^{-2}$ (30kg h^{-1}), to a depth of 10-15mm. Seeds was obtained from McDonald Seeds[2]. To ensure even coverage of the seeds and microbes, the seeds were divided into two. One half was sown in one direction and the other at right angles to the first.

The trial commenced at planting in the second week of April 2002 and was carried through the winter until September 2002.

Treatments

The antagonistic potential of experimental formulations comprising spore suspensions in inert carrier media, were determined against a water control. The treatments included *Bacillus subtilis* Ehrenberg & Cohn. B69 in talcum powder (designated *Bacillus* B69), *Trichoderma harzianum* Rifia kd in shredded wheat (designated *Trichoderma* kd) and *Gliocladium virens* Miller, Gidens, Foster & von Arx in kaolin with oak husk (designated *Gliocladium*). The product formulations are shown in Figure 6.2 and were obtained from Plant Health Products cc[3].

[2] McDonald Seeds, P.O. Box 40, Mkondeni, 3200, South Africa. Tel (+27)33 3460121

[3] Plant Health Products cc., P.O. Box 207, Nottingham Road, 3280, South Africa. Tel: (+27) 33 263 6130

A

B

C

Figure 6.2 **Dusted ryegrass seed treatments for the establishment of the grazing preference trial conducted at Cedara (April-September 2002). (A) *Bacillus subtilis* in talcum powder; (B) *Trichoderma harzianum* in shredded wheat bran; (C) *Gliocladium virens* in kaolin with oak husk.**

Initial treatments were applied as seed dustings of the spore suspensions, at planting in April 2002. A control treatment comprised uninoculated seeds. Seeds were sown by hand, after which the plots were raked to ensure good seed-to-soil contact. The newly planted area was lightly irrigated to ensure that treatments infiltrated and inoculated the soil profile. Thereafter, the area received 25mℓ water per week on a 72 hr cycle. Treatments were reapplied at 10 day intervals, as a soil drench, using 5ℓ hand watering cans (one per treatment) with a pouring nozzle head of 6.2cm X 7.8cm, with spray holes 1mm in diameter. Pure water was used as the control. Treatment application rates are shown in Table 6.1.

Table 6.1. Application rates for treatments of grazing trial to determine treatment effect on palatability and preference of grazers on ryegrass treated and untreated with biological control agents (2002)

Treatment number	Treatment name	Application rate administered	Manufacturer's application rate
1	*Bacillus* B69	8g/5ℓ	1g/1ℓ
2	*Trichoderma* kd	8g[a]/5ℓ[b]	
3	*Gliocladium*	8g[a]/5ℓ[b]	
4	Water	5ℓ	-

a At planting 8g of the biological control agents was applied as a dust seed treatment

b For a $2m^2$ area the application rate is 8g/8ℓ. This was too great a volume of water, so 5ℓ was used.

Observations and analyses

Approximately nine weeks after planting (WAP), $1m^2$ foliage samples were removed from the center of each plot to determine the effect of treatments on biomass (wet and dry weights). Wet weight was determined in field, as a representative sample ($1m^2$ foliage sample) from each plot. A mean per treatment was calculated for the wet weights (g). Representative samples from each treatment plot, were oven dried at 60^0C for 48hrs to determine dry weight (g). A mean per treatment was then calculated for dry weights . The entire field plot was then defoliated to a height of 50mm with a self-propelled sickle-bar

mower to ensure consistency for future biomass determination. To maintain pasture quality, plots were mowed a second time approximately 12 WAP and before the first grazing observations were taken. The determination of biomass, as above, was repeated again at the termination of the trial to determine the effects of grazing preference (if any) and the treatments on final wet and dry weights.

Wet and dry biomass were analyzed using correlation analysis, Quattro Pro Corel 9 (Anon, 2000a), to determine any relation between the treatments and increased weights. Bar charts were also generated. The final wet and dry weighs, as well as dry matter percentage (at trial termination) were analyzed using the graphics tool in Quattro Pro Corel 9 and analysis of variance (ANOVA) in Genstat 5 (Anon, 2000b).

The final dry biomass samples (at termination of the trial) were pooled into three treatment representative samples. These were submitted to the Cedara Feed Laboratory[4] to determine the effect of treatments on plant nutrient content.

Three observations were made of the sheep (ewes) grazing the trial area, 16 WAP (mid-winter); 20 WAP (late winter) and 25 WAP (early spring). Three sheep homogeneous in age and weight, approximately 60kgs, were starved 12hrs before the observations and then placed on the plots one hr before their grazing activities were monitored. Sheep had a choice of grazing from the 16 plots. Grazing habits of only one tagged sheep was monitored and noted before midday for a time period of four hrs, at five minute intervals. The perimeter of the trial was enclosed with orange sheep fencing to prevent grazers from wandering to neighbouring pastures. Data was analyzed using the graphics tool in Quattro Pro Corel 9 to generate bar charts and ANOVA in Genstat 5 (Anon, 2000).

[4] Cedara Feed Laboratory, KwaZulu-Natal Department of Agriculture and Environmental Affairs, Private Bag X 9059, Pietermaritzburg, 3200, South Africa. Tel: (+27) 33 3559449

6.3 RESULTS

The effect of treatments on wet and dry weights and calculated dry matter percentage of annual ryegrass at 9 WAP is summarised in Table 6.2. The ANOVA test summarised in the table shows that the treatments had no significant ($P \geq 0.05$) effect.

Table 6.2 ANOVA of mean wet weight (WW); dry weight (DW) and dry matter % (DM%) associated with treatments *Gliocladium*, *Trichoderma* kd, *Bacillus* B69 and water control on the annual ryegrass grazing trial conducted at Cedara (April - September, 2002) at nine weeks after planting

		A WW (g)	R	B DW (g)	R	(A/B)*100 = C DM (%)	R
Treatments	Water	407	3	86.8	3	21.7	3
	Gliocladium	345	1	82.2	2	23.8	4
	Trichoderma kd	454	4	93.8	4	21.3	2
	Bacillus B69	368	2	65.0	1	18.9	1
Grand mean		394		81.9		21.5	
LSD		192.6		41.84		7.07	
Degrees of freedom (residual)		9		9		9	
F value	reps stratum	1.23		0.11		1.39	
	rep. treats stratum	0.64		0.88		0.80	
Probability (P)		0.61 (NS)		0.49 (NS)		0.52 (NS)	
Standard error (s.e.)		120.4		26.16		4.42	
CV%		30.6		31.9		20.6	

R = rankings between treatments

Figure 6.3 summarizes the mean, minimum and maximum wet biomass (g) of the treated plots, 9 WAP. Figure 6.4 summarizes the mean, minimum and maximum dry biomass (g) of the treated plots, 9 WAP. The trend shown in each of the figures was that the highest mean wet and dry biomass recorded was associated with *Trichoderma* kd. The second highest wet biomass was associated with *Bacillus* B69. The water control accounted for the lowest wet biomass (Figure 6.3).

Pasture quality is expressed as dry matter percentage, calculated as a % of dry weight over wet weight. Figure 6.5 shows dry matter percentage associated with the different treatments. The water control was associated with the highest mean dry matter percentage. *Bacillus* B69 and *Trichoderma* kd treatments, associated with the highest wet biomass, reflected a low dry matter percentage.

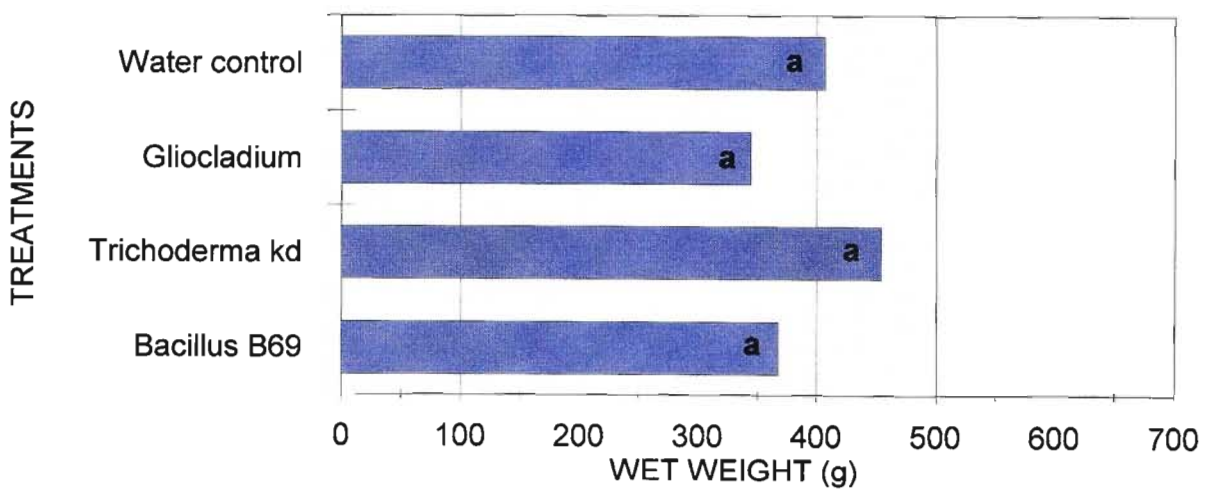


Figure 6.3 Mean wet biomass (g) nine weeks after planting; annual ryegrass grazing trial conducted at Cedara (April - September, 2000).

Note: Treatment means with similar letters are not significantly different from each other based on an LSD test at the 5% confidence level

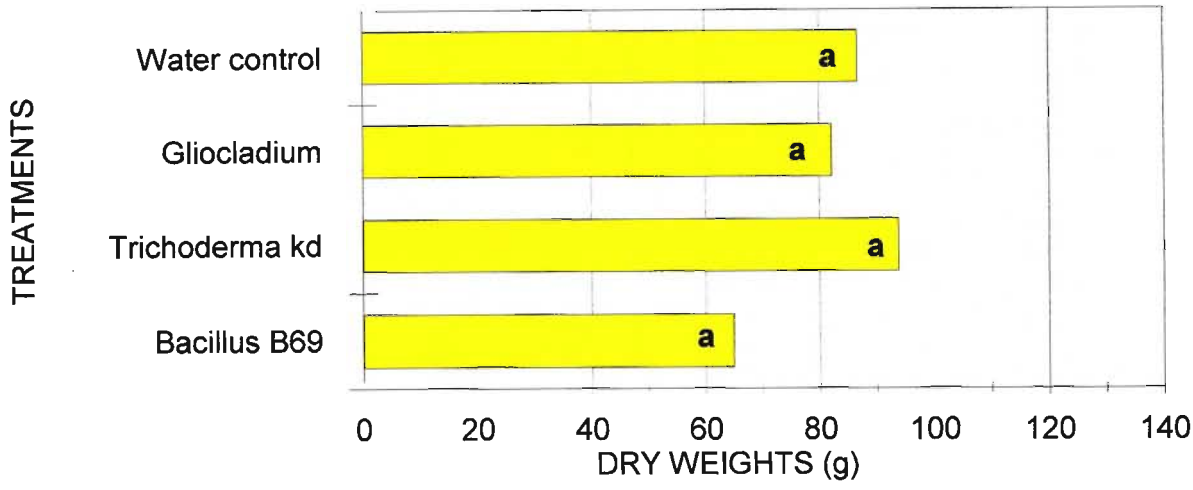


Figure 6.4 Mean dry biomass (g) nine weeks after planting; annual ryegrass grazing trial conducted at Cedara (April - September, 2002).

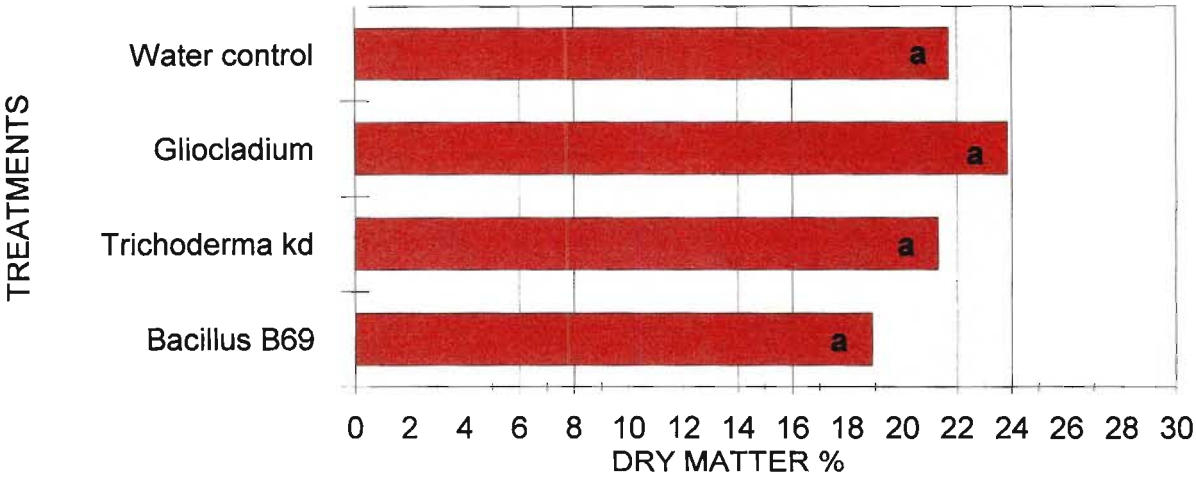


Figure 6.5 Mean dry matter percentage (dry weight/wet weight) nine weeks after planting; annual ryegrass grazing trial conducted at Cedara (April - September, 2002).

Note: Treatment means with similar letters are not significantly different from each other based on an LSD test at the 5% confidence level

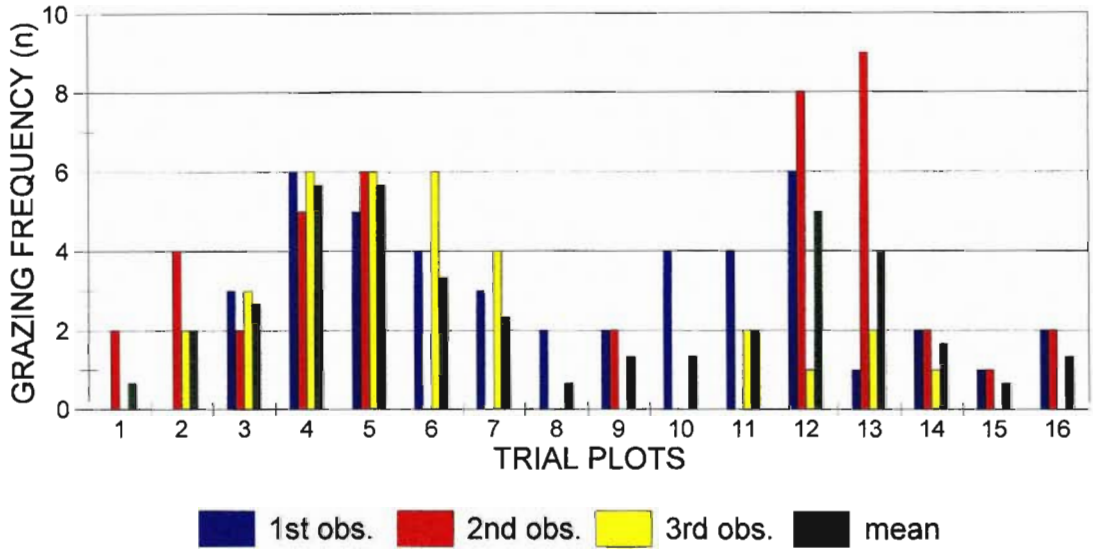
ANOVA test is summarized in Table 6.3, for each grazing observation, i.e., at 16, 20 and 25 weeks and the mean of these three observed grazing frequencies. There was no significant difference ($P \leq 0.05$) in grazing preference noted between plot treatments and grazing preferences of the ewes, indicating that the treatments had no effect on grazing selectivity.

Table 6.3 ANOVA of first, second and third grazing frequency observations and the mean grazing frequencies of treatments *Gliocladium*, *Trichoderma* kd, *Bacillus* B69 and water control on the annual ryegrass grazing trial conducted at Cedara (April - September, 2002)

		First (16 weeks) grazing frequencies	R	Second (20 weeks) grazing frequencies	R	Third (25 weeks) grazing frequencies	R	Mean grazing frequency	R
Treatments	Water	2.25	1	2.75	2	2	1	2.33	2
	Gliocladium	3.5	3	3	3	2	1	2.83	4
	Trichoderma kd	3.25	2	1.5	1	2	1	2.25	1
	Bacillus B69	2.25	1	3.5	4	2.25	2	2.67	3
Grand mean		2.81		2.69		2.06		2.52	
LSD		2.8		2.33		3.6		1.15	
Degrees of freedom (residual)		9		9		9		9	
F value	reps stratum	2.36		15.69		2.19		25.08	
	rep. treats stratum	0.56		1.37		0.01		0.58	
F Probability (P)		0.65 (NS)		0.31 (NS)		0.99 (NS)		0.64 (NS)	
Standard error (s.e.)		1.75		1.46		2.25		0.72	
CV%		62.2		54.2		109.1		28.6	

R = ranking between treatments

From the grazing observations, the sheep grazed more to one side of the 64m^2 trial area, including Plots 4, 5, 12 and 13 (Figure 6.2). The grazing pattern of ewes is represented in Figure 6.6.



Water trial plots = 1, 6, 10, 13	*Gliocladium* trial plots = 3, 7, 12, 16
Bacillus trial plots = 2, 5, 9, 14	*Trichoderma* trial plots = 4, 8, 11, 15

Figure 6.6 Grazing frequency of ewes (3 observations and the mean of these) as observed at 16 (1st observation), 20 (2nd observation) and 25 (3rd observation) weeks after planting; annual ryegrass grazing trial conducted at Cedara (April - September, 2002).

For a significant result, the mean grazing frequency would have to equal 0.31 (Highest treatment mean - Grand mean). Variation (F values) was determined for each of the small trial plots, as well as within the individual small trial plots. Greatest variation (i.e., higher F values) was associated between the trial plots (reps stratum), rather than within the treatment replication plots (rep. treatment stratum).

ANOVA revealed no significant ($P \leq 0.05$) association between the treatments applied to the annual ryegrass and an increased growth/yield response. Results of the ANOVA test are summarized in Table 6.4.

Table 6.4 ANOVA of mean wet weight (WW); dry weight (DW) and dry matter % (DM%) associated with treatments *Gliocladium*, *Trichoderma* kd, *Bacillus* B69 and water control on the annual ryegrass grazing trial conducted at Cedara (April - September, 2002) at 25 weeks after planting

		A WW (g)	R	B DW (g)	R	(A/B)*100 = C DM (%)	R
Treatments	Water	352	1	88.8	3	26.4	4
	Gliocladium	357	2	84.0	2	23.5	3
	Trichoderma kd	439	4	97.0	4	22.2	2
	Bacillus B69	380	3	65.5	1	18.6	1
Grand mean		382		83.8		22.7	
LSD		168.3		35.44		7.3	
Degrees of freedom (residual)		9		9		9	
F value	reps stratum	2.54		1.33		1.12	
	rep. treats stratum	0.83		1.22		0.75	
Probability (P)		0.51 (NS)		0.36 (NS)		0.55 (NS)	
Standard error (s.e.)		105.2		22.16		4.56	
CV%		26.7		27		21.3	

R = rankings between treatments

Although ANOVA showed non-significant results, general treatment trends are shown for the final foliage samples taken 25 WAP for wet biomass (Figure 6.7), dry biomass (Figure 6.8) and dry matter percentage (Figure 6.9). The trend of Figures 6.7 and 6.8, showed that the greatest wet and dry biomass resulted from *Trichoderma* kd, indicating that *T. harzianum* promoted plant growth.

In terms of the DM % (Figure 6.9), *Bacillus* B69 was associated with the lowest dry matter percentage, and *Gliocladium* a higher mean dry matter percentage of the microbe-based treatments, but still less than the water control.

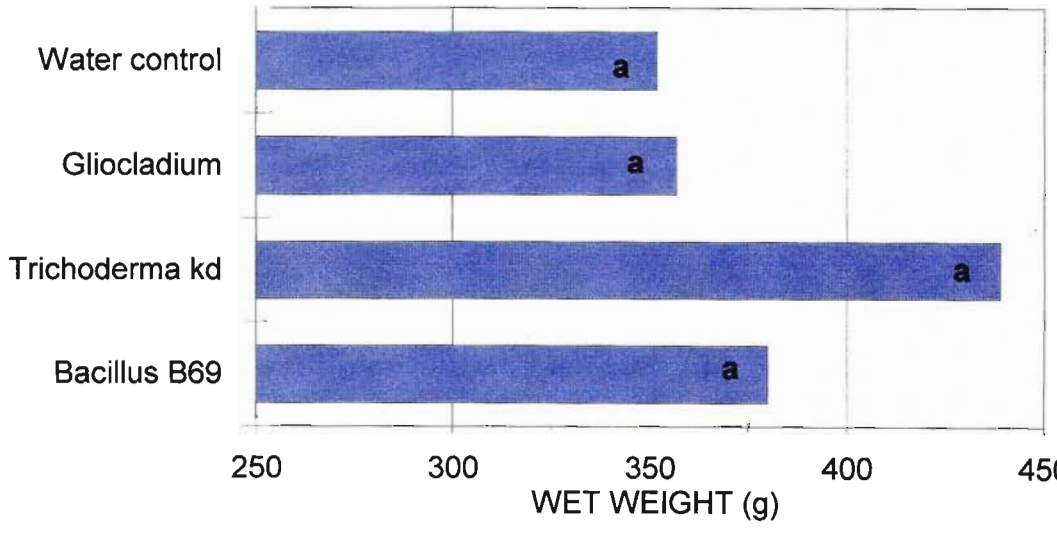


Figure 6.7 Mean wet biomass (g) 25 weeks after planting, i.e. third grazing observation; annual ryegrass grazing trial conducted at Cedara (April - September, 2002).

Note: Treatment means with similar letters are not significantly different from each other based on an LSD test at the 5% confidence level

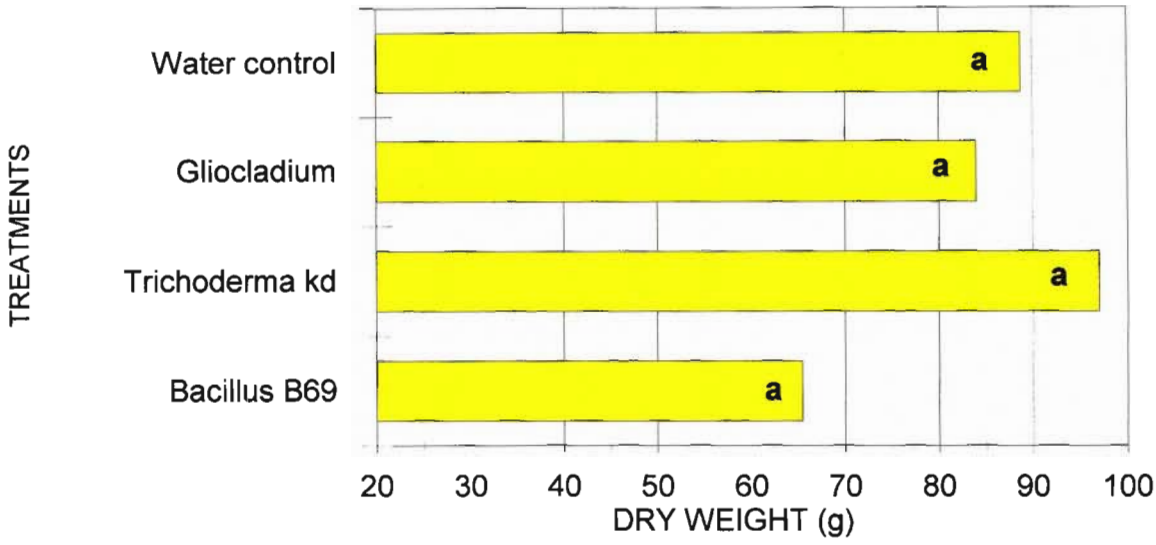


Figure 6.8 Mean dry biomass (g) 25 weeks after planting, i.e., third grazing observation; annual ryegrass grazing trial conducted at Cedara (April - September, 2002).

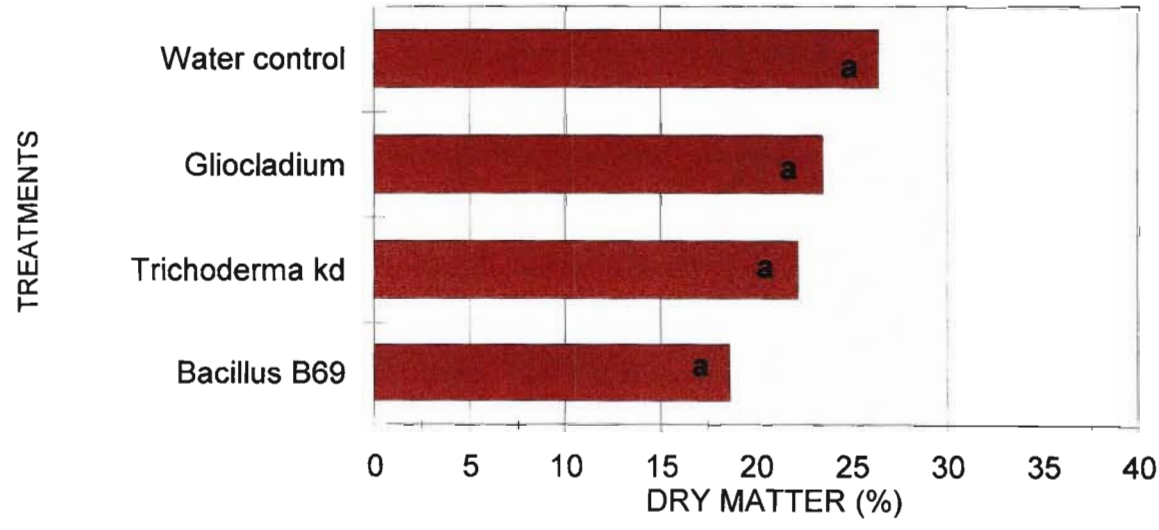


Figure 6.9 Mean percentage dry matter (dry weight/wet weight) 25 weeks after planting, i.e., third grazing observation; annual ryegrass grazing trial conducted at Cedara (April - September, 2002).

Note: Treatment means with similar letters are not significantly different from each other based on an LSD test at the 5% confidence level

Effect of treatments on nutrient content of annual ryegrass is summarized in Table 6.5. It is not the values of the analysis that are important but rather the ratings, i.e., fat content of the samples is highest with the water control, while *Trichoderma* kd had the lowest fat%. Acid detergent fibre (ADF) (a complex fiber) and neutral detergent fibre (NDF) (a more easily digestible fiber) contents are exceptionally high in comparison to the norm (Bredon, *et al.*, 1987). *Gliocladium* was associated with the greatest % NDF, while *Trichoderma* kd was associated with the highest % ADF.

Table 6.5 Summary of the effect of treatments *Gliocladium*, *Trichoderma* kd, *Bacillus* B69 and the water control, on the nutrient content and palatability of annual ryegrass foliage samples from the annual ryegrass grazing trial conducted at Cedara (April - September, 2002)

Treatments	100% Dry Matter Basis												
	Fat	Fibre		Sugar	Protein	N	Ca	Mg	K	Na	P	Zn	Cu	Mn
		ADF	NDF	NSC										
Water	2.5	42.1	65.95	11.25	14.17	0.75	0.52	0.14	1.19	0.62	0.21	37	8	95
Gliocladium	2.4	45.5	66.33	11.04	12.75	0.61	0.51	0.13	1.37	0.4	0.2	35	6	108
Trichoderma kd	1.1	48.3	64.92	8.13	13.27	0.43	0.45	0.22	1.37	0.36	0.21	37	8	115
Bacillus B69	2.3	42.9	65.45	12.08	10.7	0.35	0.35	0.2	1.07	0.52	0.18	31	8	108

ADF is Acid Detergent Fibre

NDF is Neutral Detergent Fibre

NSC is Non Structural Carbohydrates

Nitrogen (N) % is determined by dividing protein by 6.25

N is NPN which is non-protein nitrogen

Metabolizable energy is an important measure of feed quality, and can be derived from ADF% for temperate forages (Dugmore, 1995). Use of BCAs was seen to increase ADF%, which implies reduced metabolizable energy. The same trend was associated with protein content and non-protein nitrogen (NPN) % content. Differences between the control and the highest value of biocontrol treatments were very small. The highest value for non-structural carbohydrates (NSC) or sugar content were associated with *Bacillus* B69. In terms of essential minerals, the application of the BCAs decreased the calcium (Ca) and sodium (Na) by as much as 7-10%. *Trichoderma* kd accounts for high magnesium (Mg)%, potassium (K)%, phosphorus (P)%, zinc (Zn)% and manganese (Mn)%. A high K % was also associated with *Gliocladium*.

6.4 DISCUSSION

Trichoderma harzianum, *G. virens* and *B. subtilis* are naturally occurring rhizosphere antagonistic microorganisms which exhibit biocontrol potentials (Agrios, 1997). The potential of these antagonists for disease control is well known (Lo, *et al.*, 1996; Agrios, 1997; Lo, *et al.*, 1997; Krebs, *et al.*, 1998; Lewis, *et al.*, 1998; Koch, 1999; Marrone, 1999). Associated with the suppression of disease, a number of antagonists also exhibit growth promotion characteristics (Chang *et al.*, 1986; Harman, 2000).

Potential for increased pasture growth

Biocontrol treatment effects on pasture establishment rates were determined by means of foliage wet and dry biomass, 9 WAP. Statistically, treatment effects on growth (dry and wet biomass) were considered to be non-significant ($P \geq 0.05$) (Table 6.2 and 6.4). The high standard error and co-efficient of variation (CV%) of ≥20% account for great variability between trial plots, resulting in the F values being non-significant. Including an extra replicate would have increased the residual degrees of freedom to 12, where significant ($P \leq 0.05$) differences may have been noted.

In the KwaZulu-Natal Midlands a ryegrass pasture is considered to be ready for utilization at approximately 6-10 WAP. Increased wet biomass trends observed in Figure 6.3, could be attributed to increased root surface area, which is often associated with BCAs (Windham, *et al.*, 1986; Kleifeld and Chet, 1992). Increased wet biomass was more pronounced in the young pasture. In terms of the wet weights of the mature pasture (i.e., 25 WAP) (Figure 6.7), the water control accounted for the second highest wet biomass. However, *Trichoderma* kd accounted for the greatest wet biomass measured. The higher control biomass could be attributed to seasonal patterns of root growth, associated with particularly temperate grass species, where root initiation and growth are naturally more rapid in late winter and early spring (Wolfson and Tainton, 2000). Rooting depth of annual ryegrass is also increased when plants are stressed (Steynberg *et al.*, 1994) which may have occurred with the change from winter to spring. Although root depth appears to account for pasture growth determination, the differences in root length for the different treatments was not confirmed in this trial.

It must be noted that as a means of determining growth, wet weight is considered inconclusive as it is largely dependent on transpiration rates, soil moisture availability and the plant's growth stage. However, increased moisture availability is associated with an increase in cell division and thus leaf expansion (Wolfson and Tainton, 2000). This is also, however, dependent on prevailing light, nutrient availability (Eckard, *et al.*, 1995) and temperature.

Potentially, dry biomass should give a better indication of growth, as variable moisture contents are removed. Dry biomass (Figures 6.4 and 6.8), indicated that the biological control treatments accounted for lower dry biomass. The negative effect that the BCAs had on foliage growth could be attributed to photosynthates being directed for increased root growth (Wolfson and Tainton, 2000). A decrease in dry biomass can also result from an increase in germination percentage (Kapulnik, 1996; Raviv *et al.*, 1998). Higher germination percentages will result in canopy or coverage being achieved much earlier. The control in turn, due to poor germination rates, had to produce bigger plants in order to form a canopy. This would account for the greater dry biomass observed.

However, a dense canopy will result in plant shading and a lower rate of plant photosynthesis and dry matter percentage (Bartholomew, 1991). Increased germination and root growth associated with *Trichoderma* kd could account for the greater mean dry biomass. However, *Trichoderma* sp. have also been shown to increase chlorophyll content (Raviv *et al.*, 1998) thus increasing the photosynthetic rate and thus dry weight. The lower mean dry weights associated with *Bacillus* B69 and *Gliocladium*, in comparison to *Trichoderma* kd (Figure 6.8), could be attributed to higher mean grazing frequencies noted for these treatment in Table 6.3.

Potential effect on pasture grazing potentials

The association between grazing frequency and treatments applied was non-significant ($P \geq 0.05$). This was due to the high variability in the grazing frequencies of the individual plots. The non-significant association shows that the sheep displayed no preference for the treated plots over the water control plot. The BCAs therefore, had no significant impact on the palatability of annual ryegrass.

In situ grazing observations are often associated with high standard error and CV%'s rendering non-significant F values due to the unpredictability of the animals (Stevens, 2002)[5]. Grazing habits/requirements of sheep are also extremely flexible and highly dependent on not only the breed of sheep or physiological state of the sheep (Klug, *et al.*, 2000), but on pasture age, fertilization, stocking rate and prevailing environmental conditions/seasons. The ewes observed in this trial were seen to be influenced by water availability, temperature and distracting activities outside the study area. An indication of grazing preference could have been better determined by removing herbage and feeding the sheep within the confined area of enclosed pens. In terms of stocking rate, Sevi *et al.* (1999) suggest a flock size of six or more sheep to be used in grazing behaviour observations. Here only three sheep were observed, of which only one's behaviour was recorded.

[5] Stevens, K. 2002. Cedara Biometrician. Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200, South Africa. Tel: (+27) 33 3559449

Drying plant material extracts moisture. In terms of grazing potential, it is this moisture content which influences the grazing potential (Meissner, 1996). The BCA treatments showed a potential trend of decreased dry matter percentage in comparison to the water control (Figures 6.5 and 6.9), thus potentially reducing dry matter intake and, therefore, animal performance. Dry matter percentage is, however, largely dependent on the frequency and severity of defoliation (Bartholomew, 1991). A poor, unpalatable, dry grass will also show a high dry matter percentage, and thus dry matter percentage was considered a poor means for measuring pasture productivity.

The nutritional requirements of grazers is variable (de Villiers, 1991). In general, a well maintained pasture should provide a good nutrient source for grazers. In terms of improving the nutritional value of pasture species associated with the amendment of antagonistic microorganisms. The results obtained in Table 6.5 show no distinct trend associated with a single treatment. Application of BCAs was associated with lower plant fat, protein (which is dependent on N), NPN, Ca and Na percentages. This reduction could be due to the microorganisms utilizing the elements for themselves (Lo, 1998). It must also be noted that Italian/annual ryegrass is not only grown as a pure stand but may be mixed with white clover to increase palatability and nutritional status of the pasture (Bartholomew, 2000).

Concluding remarks

From this trial, it was concluded that the sheep showed no preferential grazing for pastures treated with BCAs. The concerns raised by the farmers in the pasture production survey (1999/2000) over the potential animal health risks associated with the use of BCAs on pastures to be grazed were unfounded as no animal mortalities or illnesses were noted for the trial duration. In terms of increased palatability and nutritional value, biological control treatments had no significant effect. Increased plant growth was observed, *T. harzianum* showing the greatest influence on grass growth. However, this was also non-significant. A better data set by means of more frequent measurements and more than one sample per treatment plot will have decreased the variability that existed in terms of the measured biomass (wet and dry weights) between plots.

6.5 REFERENCES

Agrios, G. 1997. Plant Pathology, 4th edition. Academic Press, California: United States of America.

Anon, 2000a. Corel Quattro Pro 9. Release 9.0.0.738. Corel Corporation and Corel Corporation Limited.

Anon, 2000b. Genstat for Windows. Release 4.2, 5th edition. VSN International Ltd, Oxford: United Kingdom.

Bartholomew, P.E. 1991. Principles of pasture utilization. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Bartholomew, P.E. 2000. The management of planted pastures. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 233-255.

Bredon, R.M., P.G. Steward and T.J. Dugmore. 1987. A manual on the nutritive value and chemical composition of commonly used South African farm feeds. Department of Agriculture and Water Supply. Pretoria: South Africa.

Chang, Y.C., Y.C. Chang, R. Baker, O. Kliefeld and I. Chet. 1986. Increased growth of plants in the presence of the biological control agent, *Trichoderma harzianum.* Plant Disease **70:** 145-148.

Curl, E.A. and B. Truelove. 1986. The rhizosphere. Springer-Verlag, Berlin: Germany.

de Villiers, J.F. 1991. Elementary concepts of sheep nutrition. In: Agricultural Production Guidelines for KwaZulu-Natal: Sheep in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Dugmore, T.J. 1995. The chemical analysis of feeds. In: Agricultural Production Guidelines for KwaZulu-Natal: Dairying in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Eckard, R.J., P.E. Bartholomew and N.M. Tainton. 1995. The yield response of annual ryegrass (*Lolium multiflorum*) to varying nitrogen and fertilizer application strategies. South African Journal of Plant Soil **12:** 112-116.

Harman, G.E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease **84:** 377-393.

Kapulnik, Y. 1996. Plant growth promotion by rhizosphere bacteria. In: Y. Wisel, A. Eshel and U. Kafkafi (eds). Plant roots: the hidden half, 2nd edition. Marcel Dekker, New York: United States of America. p. 769-781.

Kleifeld, O. and I. Chet. 1992. *Trichoderma harzianum*: interaction with plants and effect on growth response. Plant and Soil **144:** 267-272.

Klug, J.R., J.M. van Heerden and A.W. Lishman. 2000. Fodder production planning and livestock production systems. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 313-318.

Koch, E. 1999. Evaluation of commercial products for microbial control of soil-borne plant diseases. Crop Protection **18:** 119-125.

Krebs, B., B. Höding, S. Kübart, M. Alemayehu Workie, H. Junge, G. Schmiedeknecht, R. Grosch, H. Boschow and M. Hevesi. 1998. Use of *Bacillus subtilis* as a biocontrol agent: activities and characterization of *Bacillus subtilis* strains. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **105:** 181-197.

Lewis, J.A., R.P. Larkin and D.L. Rogers. 1998. A formulation of *Trichoderma* and *Gliocladium* to reduce damping-off caused by *Rhizoctonia solani* and saprophytic growth of the pathogen in a soilless mix. Plant Disease **82:** 501-506.

Lo, C.T., E.B. Nelson and G.E. Harman. 1996. Biological control of turfgrass diseases with a rhizosphere competent strain of *Trichoderma harzianum.* Plant Disease **80:** 736-741.

Lo, C.T., E.B. Nelson and G.E. Harman. 1997. Improved biocontrol efficacy of *Trichoderma harzianum* 1295-22 for foliar phases of turf diseases by use of spray applications. Plant Disease **81:** 1132-1138.

Lo, C.T. 1998. General mechanisms of action of microbial biocontrol agents. Plant Pathology Bulletin **7:** 155-166.

Lynch, J.M. 1992. Environmental implications of the release of biocontrol agents. In: E.C. Tjomas; G.C. Papavizas and R.J. Cook (eds). Biological control of plant diseases: progress and challenges for the future. Plenum Press, New York: United States of America.

MacVicar, C.N. 1991. Soil classification. A taxonomic system for South Africa. Memoirs on the Agricultural Resources of S.A. No. 15. Department of Agricultural Development, Pretoria: South Africa. p. 257.

Marrone, P.G. 1999. Microbial pesticides and natural products as alternatives. Outlook on Agriculture **28:**149-154.

Meissner, H.H. 1996. A comparison of Italian ryegrass (*Lolium multiflorum*) cultivars Exalta and Midmar with respect to their nutritive value to sheep. South African Journal of Animal Science **26:** 37-41.

Raviv, M., B.Z. Zaidman and Y. Kapulnik. 1998. The use of compost as a peat substitute for organic vegetable transplant production. Compost Science and Utilization **6:** 46-52.

Sevi, A, D. Casamassima and A. Muscio. 1999. Group size effects on grazing behaviour and efficiency in sheep. Journal of Range Management **52:** 327-331.

Steynberg, R.E., P.C. Nel and N.F.G. Rethman. 1994. Soil water use and rooting depth of Italian ryegrass (*Lolium multiflorum* Lam.) in a small plot experiment. South African Journal of Plant and Soil **11:** 80-83.

Windham, M.T., Y. Elad and R. Baker. 1986. A mechanism of increased growth induced by *Trichoderma* spp. Phytopathology **76:** 518-521.

Wolfson, M.M and N.M. Tainton. 2000. Grasses: The morphology and physiology of the major forage plants. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 14-34.

CHAPTER 7

OVERVIEW AND FUTURE DIRECTIONS OF BIOLOGICAL CONTROL ON TURF AND PASTURE GRASSES

7.1 GENERAL OVERVIEW AND DISCUSSION OF BIOLOGICAL CONTROL

Pasture and turf production are intensive, often monocropped systems requiring high production rates for quick recovery. High growth rates must therefore be maintained. However, achieving this comes at a high monetary cost. For economically sustainable production, high costs associated with pasture establishment and maintenance must be measured against pasture productivity in terms of animal performance (Whitehead and Dunn, 1991). In terms of turf, establishment and maintenance costs are measured by the provision and utilization of a good playing surface.

Grass management entails an integration of cultural practices and agrochemicals to sustain healthy plant growth. A grassland is an ecosystem of interactions between abiotic and biotic factors, biotic factors including pathogen and antagonist interactions for biological control. Of late, sensitivity towards the use of agrochemicals in public areas provides the incentive for integration of biological control into production systems (Harman, 2000). In developing countries such as Africa, "natural systems" could be exploited as an alternative to the use of expensive agrochemicals.

Results obtained from this evaluation of BCAs (isolates *Trichoderma harzianum* and *Bacillus subtilis*), showed their potential for disease control and plant growth stimulation. The strain of *Gliocladium virens,* applied as a soil drench to turfgrass varieties, showed growth stimulation. It is important to always keep in context that the isolation, manipulation and *in vitro* testing of a potential antagonistic strain does not necessarily

mean a new BCA, rather it is only the start to a process of further evaluation (Dent, 1993).

The aim of this research was to identify the stability of the amended microbes *in vivo.* However, based on inconsistencies in disease control and growth stimulation observed in this thesis further field testing is required. Being living organisms, a number of factors affect microbial activity and success of biological control. Determination of these factors and their management or manipulation to rather stimulate microbe activity is vital. It was unfortunate that disease control and growth stimulation were associated with such a high percentage of non-significant results, as the "failures" and variability of results limit the adoption of biological control on a commercial scale. As mentioned in the literature review (Chapter 1), further development of biological control is in an integrated management system. This applies not only to the use of BCAs together with agrochemicals and cultural practices, but also in the use of two or several compatible BCAs (Dandurand and Knudsen, 1993). Integration potential of BCAs could be an approach to the future of biological control for plant health promotion.

Experimental errors must be reduced. Therefore, sample size and replicate numbers used in the trials must be increased to reduce variability. Disease assessment and growth measurement methods must also be reassessed to reduce personal bias. Although, it is common practice to use a disease rating scale to determined disease progress, rating scales leave a very grey area of definition between defined scales. Better disease definition could be achieved by means of digital analysis, defining the leaf area infected versus uninfected area. Application will, however, be determined by sample numbers per plot and time constraints. Colour analysis, through the use of digital photographs, could be applied to determine soil cover, i.e., establishment rates and therefore plant growth stimulation.

7.2. POTENTIAL FOR DISEASE CONTROL

7.2.1 INFLUENCE OF MANAGEMENT PRACTICES AND PLANT HEALTH

Stressed plants are more susceptible to disease, particularly low sugar diseases (Agrios, 1997). Management principles must therefore promote optimum plant growth conditions. The production surveys (Chapter 3) showed management practices to have a direct impact ($P \leq 0.05$) on not only disease incidence but insect occurrence and weed growth also, these impacting on plant health status.

For example, the application of nitrogen (N) is often listed for disease control, the hypothesis being that due to growth stimulation the plant will "out-grow" disease symptoms. Nitrogen applications do, however, also have the potential to increase disease incidence by promoting "soft", nutritious growth and tissues that are more susceptible to pathogen attack. Disease occurrence associated with the implementation of cultural control measures in turf production in Chapter 3 was significant ($P \leq 0.1$).

In terms of pasture production, grazing management decreased foliar disease occurrence significantly ($P \leq 0.05$). However, grazing too hard will impact negatively on disease occurrence due to a higher plant stress incidence. Where different animal classes graze the same pasture, disease incidence increases significantly. Grazing has its place in disease control in terms of having the potential to remove the pathogen. Integration with a BCA would also have the potential for reduced disease incidence, while stimulating regrowth after grazing. Use of BCAs (*Bacillus, Trichoderma* and *Gliocladium* isolates) on pasture grasses was shown to have no influence on grazing preference and showed no pathogenicity to the grazers (Chapter 6). Whether this preference is limited to specific grazing classes (in this research sheep ewes were observed) or due to grazing *in situ,* would have to be determined. Grazing observations are, however, better managed by means of feeding animals cut samples from the various plots, and then observing for any preferences (Stevens, 2002)[1].

[1] Stevens, K. 2002. Cedara Biometrician. Department of Agriculture and Environmental Affairs, Private Bag X9059, Pietermaritzburg, 3200, South Africa. Tel: (+27) 33 3559449

7.2.2 DISEASE CONTROL ON *PENNISETUM CLANDESTINUM*

The application of BCAs is still considered a measure for the re-establishment of a "biological balance" between antagonistic and pathogenic microbes. *In vitro* and *in vivo* trials showed that the potential for control of Helminthosporium leaf spot is achieved with *B. subtilis* and *T. harzianum*-based BCAs. Correct application rates of these BCAs is also important for the level of disease control achieved. From the trials it was determined that the manufacturer's recommended dosage rate showed greater disease control ($P \leq 0.05$) in comparison to the zero application (control).

The potential influence of climatic conditions on microbial activity was considered for the duration of the trial, but little attention was given to the presence of microfauna already established in the growing medium and their potential pathogenicity towards the antagonists. Kleifeld and Chet (1992), go as far as to suggest that disease control (and possible plant growth stimulation) would be better achieved in a pathogen free soil. Predetermination of existing antagonists in the soil and a means by which to augment their numbers for disease control and growth stimulation would also prove advantageous.

BCAs were applied as drench treatments for the control of a foliar disease. It was also hypothesised that some of the antagonists washed down into the thatch layer. Thatch colonization would have decreased disease inoculum levels, decreasing the apparent rate of disease infection. The microbes may also have penetrated the soil to colonize the plant's rhizosphere controlling disease by means of systemic acquired resistance (Harman, 2000). This was, however, not confirmed by means of reisolation and microscopic confirmation. Understanding mechanisms of disease control associated with the application of BCAs is important for understanding the concept of biological control and "conditions" required for the mechanism to be employed or manipulated for optimum control to be achieved.

Integration of BCAs for broader disease control should also be explored. This is largely based on the compatibility of two or more microbes (Harman, 2000). *Trichoderma* and *Bacillus* spp. were shown to be compatible *in vitro* by means of a dual agar antagonism test (Chapter 4). *In vivo*, the level of disease control achieved between the two was seen

to be variable. Thus, the application of both microbes will potentially offer a wider range for disease control. The question in terms of compatibility is also raised for the simultaneous use of fungicides. Several strains of BCAs (including *T. harzianum*) exist which are fungicide resistant. Although *Bacillus*- and *Trichoderma*-based treatments were tested against PUNCH XTRA®, the potential compatibility of PUNCH XTRA® and the microbial treatments was not predetermined. This would require further investigation.

Another question that requires verification is the possibility of pathogens developing resistant strains to the antagonists as they do to fungicides. Correct application rates and timing of application of BCAs must be determined by the manufacturers and followed by users of the products. The potential for pathogen resistance against BCAs, however, is considered minimal due to the multiple modes of action of microbes for disease control and plant growth stimulation.

Host specificity of antagonists in terms of rhizosphere colonization may also be a factor affecting disease control (Koch, 1999). Host plant interactions with amended antagonistic microbes requires further investigation. Whether to apply "processed" antagonistic microbes or rather stimulate indigenous antagonistic populations already present in the soil through soil environment manipulation (Papavizas, 1985), is a further consideration for future investigation.

7.2.3 POTENTIAL FOR DISEASE CONTROL ON THE PHYLLOPLANE

Further research is required to determine the potential phylloplane effects of amended antagonists on pathogens. Scanning electron microscopy did show amended *Trichoderma* sp. persistence on the phylloplane when observing *Bipolaris* sp. lesions, this suggesting potential phylloplane interactions for pathogen control. For example, *T. harzianum* applied in a granular and spray application has proved an effective control measure against three foliar diseases associated with creeping bentgrass (Lo *et al.*, 1997). Literature states that disease control on the phylloplane is common (Spurr and Knudsen, 1985). Phylloplane competence and factors affecting persistence and activity require further investigation.

7.2.4 DISEASES OF FUTURE IMPORTANCE, AS OUTLINED BY PRODUCTION SURVEYS (1999/2000)

Potential control of Helminthosporium leaf spot with the application of BCAs was determined (Chapter 4). This is a commonly encountered disease which appears to be of little threat to pasture grasses in terms of grazing potential. Although disease incidence may be high, the actual intensity of disease is low (Lam and Lewis, 1983). In the turfgrass industry where aesthetic appearance is of utmost importance, Helminthosporium- incited diseases pose a serious problem and control measures must be implemented. The potential for disease control achieved with the application of *Bacillus* sp. and *T. harzianum* was shown in the results obtained from the trials. Disease control achieved with *T. harzianum* showed no significant difference ($P \geq 0.05$) to the systemic fungicide PUNCH XTRA®.

For future research, control of kikuyu yellows and ryegrass blast in the KwaZulu-Natal Midlands must be considered. Ryegrass blast appeared to have considerable effect in the first season only. The pathogen has the potential to be equally destructive on both turf and pasture grasses, causing extensive production losses to newly-established areas (Uddin *et al.,* 1999). Disease occurrence is largely attributed to weather conditions (Smiley *et al.,* 1992). In KwaZulu-Natal, the 2000 season experienced high rainfall resulting in humid conditions (90%) and extended leaf wetness periods (6-16 hrs). Although the pasture production survey (Chapter 3) reveals only 3.88% of farmers encountering blast, results were from the early season of 2000 (April/May).

From the pasture survey, kikuyu yellows accounted for the highest disease incidence (34.95%). Kikuyu yellows also attributed the greatest significance ($P \leq 0.05$) in terms of the management practices and disease incidence. Kikuyu yellows is also associated with turf grasses, accounting for 5.66% of diseases recorded in the turf production survey (1999/2000). Kikuyu yellows detracts from the aesthetic value of a turf stand because the grass is severely yellowed and uneven due to dead or thinned patches. In a pasture, kikuyu yellows decreases productivity, but has no apparent influence on the palatability of the pasture as it is still grazed (Lenné, 1994). However, being a new disease, many

farmers are cautious about grazing the infected pasture. Available literature is largely based on Australian pasture conditions and disease severity. At present little information is available on the disease concerning South African farming conditions.

It is suspected that severe yellowing of a grass may be associated with N deficiency. Increased N availability is associated with the use of *Trichoderma harzianum* (Harman, 2000). However, where proved that yellowing was induced by something other than a nutrient deficiency, pathogen occurrence can be suspected (Fulkerson and Wong, 1996). If the causal agent is suspected to be a fungal, isolation of the potential pathogen from an infected area followed by a successful Koch's postulate would prove fungal pathogens to be the causal agent (Wong, 2000[2]).

Future research for control of kikuyu yellows and blast would comprise a comparison of the level of control with *Bacillus*- and *Trichoderma*-based biological control products, nitrogen applications (specifically for kikuyu yellows) and various fungicides.

7.3 POTENTIAL FOR GROWTH STIMULATION

7.3.1 GROWTH STIMULATION ASSOCIATED WITH DISEASE CONTROL

The association between disease occurrence and plant growth potential was found to be correlated, in that the highest dry matter % of kikuyu was associated with the full dose of microbial treatments for control of Helminthosporium leaf spot (Chapter 4).

From the pasture and turf production surveys (1999/2000), mention was made of seedling diseases which decrease seed germination, grass establishment and percent cover achieved. Although there are a number of mechanisms of action implemented by BCAs (Chapter 1), colonization of the rhizosphere with an antagonist population to reduce soilborne pathogen infection is a proposed mechanism of enhanced plant growth.

[2] Dr P.T.W. Wong. Plant Pathologist. New South Wales Agriculture. wongp@agric.nsw.gov.au

An understanding of activities on the rhizosphere is, therefore important for understanding increased plant vigour (Dandurand and Knudsen, 1993).

The establishment of a suppressive soil is slow (Kerry, 1992). Consequently, disease control and/or growth stimulation are not immediately expressed. Here, integration of BCA applications with a fungicide for initial disease control and the BCA as a pathogen population "maintenance" approach would be ideal. Compatibility of BCAs and fungicides as a seed treatment at planting is a subject for future research.

7.3.2 INCREASED GERMINATION AND PLANT ESTABLISHMENT

Plant growth is influenced by a number of factors, which potentially also effect microbial activity. Inconsistency in growth stimulation observed with the application of microbial treatments *in vivo* on turf grasses (Chapter 5) questions significant growth stimulation observed.

Variability could be attributed to climatic conditions, particularly with lower temperatures associated with the trial period (Autumn/Winter). Establishing a similar trial in September running until mid-summer would verify the influence of lower temperature on microbial activity and plant growth responses observed. Irrigation removed the influence of water stress as a limiting factor for microbial activity. MICROBOOST® applied as an activator to microbial treatments, was associated with the microbes having a significant ($P \leq 0.05$) effect on increasing root growth both *in vitro* and *in vivo.* However, little effect was noted for increased microbial activity in stimulating germination and establishment rates. Factors such as germination viability of seed must also be considered for further studies, as well as the effect of planting depth on germination % and thus establishment rates.

Enhanced root development, induced by microbes, increases the absorptive area for water and nutrient uptake (Harman, 2000). Increased nutrient uptake is also attributed to an increase in root cell permeability, also induced by microbes (Brown, 1974). Microbes are known to increase nutrient availability to plants, in particular nitrogen (Harman, 2000). In all grass swards, 50-70% of all applied N fertilizer is normally utilized

by the plant (Miles and Manson, 2000). Increased root growth was shown in Chapter 5, future studies would be required to determine if increased utilization of applied fertilizers associated with the amendment of microbes resulted in the increased growth. Certain microbial strains are known to stimulate production of plant growth regulators upon colonization of the host (Harman, 2000). This too would require future investigation.

Variability in growth stimulation observed may be attributed to the grass types established. Although grasses established were cool-season varieties, differing growth responses to microbial treatments were also observed. This raises the question as to host specificity in terms of establishment in the rhizosphere. *In vitro* perennial ryegrass showed improved growth. *In vivo*, fescue which took longest to germinate, showed increased growth associated with microbial treatments when compared to the untreated control. Slower germination of fescue would allow for better establishment of antagonists in the soil prior to rhizosphere colonization upon seed germination. As already mentioned, establishment of a suppressive soil is slow (Kerry, 1992). This was particularly evident *in vitro* where for both trials little effect was noted in terms of improved germination and plant emergence associated with the microbial treatments, but at trial termination microbe treatments were associated with greater growth. Perhaps the initial microbial amendment was at a rate too low for successful colonization and only upon re-application did the antagonist population become sufficient for growth responses to be noted.

Stimulated growth associated with microbial treatments could have many commercial applications. Increased root growth observed would be associated with an increase in shoot growth (Wolfson and Tainton, 2000), which in turn, would provide more grazing material. Because plants would have a more extensive root system, recovery rates would be shorter, reducing the pasture rest period before the next grazing. Increased root growth should also be associated with increased nutrient uptake and thus improved nutritional value as well. In terms of turfgrasses, sod-farming is becoming more of a viable farm enterprise (Adrian *et al.*, 1996). Increased plant growth would reduce potential plant stress due to relocation of the sods and would potentially increase re-

establishment rates. Increased growth rates of grasses would also be advantageous in the rehabilitation rates of mines or dunes. Amendment of antagonistic microbes would create a suppressive soil which would promote further plant succession.

In general, a greater understanding of what happens to microbes upon introduction into the soil environment, as well as the time period required for rhizosphere colonization and interactions that occur on the rhizosphere that induce the stimulated growth response, is required. There are numerous speculations with little factual data. Information as to the effect of specific climatic conditions, indigenous soil microbes, the quantity and state of activity at which the antagonistic microbes are amended and the host plant itself, are required.

7.3.3 GROWTH STIMULATION EFFECTS ON QUALITY OF GRAZING MATERIAL

Pastures in KwaZulu-Natal can be utilized 6-10 weeks after planting. Due to growth stimulation associated with BCA use, this period could potentially be reduced. Increased wet biomass (g) was associated with BCA applications to *Lolium multiflorum* (Chapter 6). This was attributed to increased root growth and therefore potentially increased water and nutrient absorption, which in turn increased foliage. Increased root growth was, however, not confirmed. Analysis of dry matter percentage attributed low dry matter to the use of BCAs. Moisture content of BCA treated plants was, however, high increasing grazing potential (Meissner, 1996). However, no preferential grazing was observed in the trial.

It is often thought that increased plant growth may be associated with decreased nutritional value, as the plant directs photosynthates into growth. Grazing observations and nutritional analysis of *L. multiflorum* established with BCAs revealed no negative effect on palatability associated with increased growth rates. In terms of increased nutritional status, results showed a decrease in nutritional value associated with BCAs, but this was non-significant ($P \geq 0.05$) and would require further investigation.

In terms of increasing nutritional value of ryegrass, it is common practice to establish the pasture with leguminous plants, such as clover, to increase overall palatability and nutritional value (Bartholomew, 1991). Legume seed require inoculation with mycorrhizal bacteria *Rhizobium* sp., for effective nodulation and biological nitrogen fixation. Survival of the inoculated bacteria is affected by a number of factors, one of which is antagonism between the rhizobium bacteria and other microorganisms present in the soil (Date and Brockwell, 1978). Potential effects of the amended microbe comprising the biological control product, in this instance *B. subtilis*, *T. harzianum* and *G. virens* would have to be observed for compatibility with *Rhizobium* sp.

Small quantities of *Trichoderma*, *Bacillus* and *Glomus intraradices* (a mycorrhizal fungus), amended at the time of seeding of tomato transplants provided season-long benefits in terms of improved plant health and yields (Harman, 2000). Thus, potential for microbe compatibility exists, the *Trichoderma* and *Bacillus* sp. providing control against soilborne pathogens and promoting plant growth while, the mycorrhizal microbe forms a symbiotic relationship with the host plant promoting plant growth.

7.3.4 POTENTIAL DISADVANTAGES ASSOCIATED WITH GROWTH STIMULATION

Increased weed growth was associated with the application of BCAs, although weed growth was not considered too severe. Weed occurrence impacts greatest on an establishing pasture (Drewes, 2000), and thus a weed-free seedbed is vital. Land preparation techniques, such as pre-irrigation to promote weed growth and use of post-emergent herbicides are essential for optimum weed control.

Weed control is achieved by mowing and grazing management (Emmons, 1995; Drewes, 2000). However, herbicides still dominate weed control in agricultural and turfgrass production (Chapter 3). Biological control has shown promise in the control of dicotyledonous weeds, displaying no pathogenicity to grasses. Future studies in weed control, include the inoculation of weeds with pathogens, specifically *Sclerotinia sclerotiorum* and *Pseudomonas syringae* pv. *tagetis*, to induce disease symptoms specific to the weed type, stressing the weed plants and reducing their competitive ability

(Neal, 1996). The potential for an alternative to weed control therefore exists.

This emphasises the potential pathogenic effect of the amended *Trichoderma* and *Bacillus* sp. on existing antagonists. Problems, however, lie in the identification of the antagonists, verifying their effects within the system and understanding the mechanisms of growth stimulation/disease control and how this can be manipulated to an optimum. Lastly, perhaps most importantly, factors affecting persistence and efficiency of antagonistic microbes must be investigated.

Increased plant growth, specifically in turf management, is not considered advantageous in terms of increased mowing frequencies, although it is common practice in the summer to increase mowing frequency to daily. Because the application of microbes is associated with increased plant vigour, the stand will comprise hardier plants with extensive root systems. Thus a lower mowing height should have little impact on even coverage being maintained with reduced dieback (Emmons, 1995). Increased plant vigour will also potentially reduce disease incidences from occurring, as is commonly associated with turfgrasses due to the stresses imposed by intense utilization. Application of microbes will depend largely on management practices already in place. Although plant health will be improved, increased vigour may prove a disadvantage.

7.4 PREDICTED FUTURE OF BIOLOGICAL CONTROL

Already BCAs are proposed for potential disease control and growth stimulation of turfgrasses in South Africa (Tainton and Klug, 2002). In Chapter 1, it was also determined that organic farming has increased interest in biological control. This demand for a natural means of production emphasises the need for biological control (Harman, 2000).

The survey revealed that farmers control pasture diseases with fungicides. The fungicides used by the farmers (Table 3.3) were, however, broad spectrum fungicides. Biological control agents would offer a less "harmful" means of disease control, while simultaneously stimulating plant growth.

In general, there was a statistically significant ($P \leq 0.1$) association between increased public knowledge about commercially available BCAs and the advantages associated with their application and implementation of biological control. The future of biological control therefore lies in increasing public awareness about BCAs. Qualms such as the cost per hectare/application must be quantified, frequency of application and optimum method of use, safety of applying live microbes to public areas and pastures researched and in general, the identification of specific factors affecting the mechanism of action implemented by the BCA determined for predictable responses. Future success of biological control is dependent on the relationship that can be forged between researchers (academics) and farmers or groundsmen (who would benefit the use of biological control). Increased public knowledge through literature, production courses and even information days should introduce biological control as a promising future for improved plant vigour. This is dependent also on a mindset change of managers, moving away from the known and reliable effects of agrochemicals to the unknown and possible more management intensive application of biological control.

As concluded by Thomas and Willis (1998): "Pest problems are growing worldwide. International trade is increasing the movement of species, and heightening environmental concerns have increased the interest in biocontrol in agriculture, conservation and the preservation of biodiversity. The need and value of biocontrol is therefore increasing ".

7.5 REFERENCES

Adrian, J.L. , P.A. Duffy and W.M. Lloyd. 1996. Turfgrass-sod: a viable farm enterprise. Journal of Production Agriculture **9:** 276-283.

Agrios, G. 1997. Plant Pathology. 4th edition. Academic Press, California: United States of America.

Bartholomew, P.E. 1991. Principles of pasture utilization. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg: South Africa.

Blakeman, J.P. 1985. Biological succession of leaf surface microorganisms in relation to biological control. In: C.E. Windels and S.E. Lindow (eds.). Biological control on the phylloplane. American Phytopathology Society, Minnesota: United States of America. p. 6-30.

Brown, M.E. 1974. Seed and root bacterization. Annual Review of Phytopathology **12:** 181-197.

Dandurand, L.M. and G.R. Knudsen. 1993. Influence of *Pseudomonas fluorescens* on hyphal growth and biocontrol activity of *Trichoderma harzianum* in the spermosphere and rhizosphere of pea. Phytopathology **83:** 265-270.

Date, R.A. and J. Brockwell. 1978. *Rhizobium* strain competition and host interaction for nodulation. In: J.R. Wilson (ed.). Plant relations in pastures. CSIRO, New South Wales: Australia. p. 202-216. Cited by: V.D. Wassermann. 2000. Legume nodulation and inoculation: Establishment of pastures. In: N.M. Tainton (ed.). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 177-179.

Dent, D.R. 1993. The use of *Bacillus thuringiensis* as an insecticide. In: D.G. Jones (ed.) Exploitation of microorganisms. Chapman & Hall, London: United Kingdom. p. 19-32.

Drewes, R.H. 2000. Establishment of pastures: semi-arid regions. In: N.M. Tainton (ed). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 175.

Emmons, R.D. 1995. Turfgrass science and management. 2nd edition. Delmar Publishers, New York: United States of America.

Fulkerson, B. and P. Wong. 1996. Kikuyu yellows (*Verrucalvus flavofaciens*) infection of kikuyu grass pastures. Research to farm. NSW Agriculture: Dairy Research and Development Corporation, Australia.

Harman, G.E. 2000. Myths and dogmas of biocontrol: changes in perceptions derived from research on *Trichoderma harzianum* T-22. Plant Disease **84:** 377-393.

Kerry, B.R. 1992. Biological control of soil-borne pests and diseases. In: Biological control and integrated crop protection: towards environmentally safe agriculture. Pudoc Scientific Publishers, Wageningen: Holland. p.117-123.

Kleifeld, O. and I. Chet. 1992. *Trichoderma harzianum*: interaction with plants and effect on growth response. Plant and Soil **144:** 267-272.

Koch, E. 1999. Evaluation of commercial products for microbial control of soil-borne plant diseases. Crop Protection **18:** 119-125.

Lam, A. and G.C. Lewis. 1983. Chemical control of foliar diseases of perennial ryegrass (*Lolium perenne* L.) and their effects on yield and quality of the crop. Crop Protection **2:** 75-83.

Lenné, J.M. 1994. Diseases of other pasture grasses. In: J.M. Lenné and P. Trutmann (eds.). Diseases of tropical pasture plants. CAB International, Wallingford: United Kingdom. p. 169-194.

Meissner, H.H. 1996. A comparison of Italian ryegrass (*Lilium multiflorum*) cultivars Exalta and Midmar with respect to their nutritive value to sheep. South African Journal of Animal Science **26:** 37-41.

Miles, N. and A.D. Manson. 2000. Nutrition of planted pastures. In: N.M. Tainton (ed.). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 186.

Neal, J.C. 1996. Biological control of dicot weeds in turf. Abstract from Cornell Community: Conference on Biological Control. April 11-13, 1996, Cornell University, Ithaca New York: United States of America.

Smiley, R.W., P.H. Dernoeden and B.B. Clarke. 1992. Compendium of turfgrass diseases, 2nd edition. American Phytopathology Society, Minnesota: United States of America.

Spurr, H.W. and G.R. Knudsen. 1985. Biological control of leaf diseases with bacteria. In: C.E. Windels and S.E. Lindow (eds.). Biological control on the phylloplane. American Phytopathology Society, Minnesota: United States of America. p. 45-62.

Tainton, N.M. and J. Klug. 2002. The cricket pitch and its outfield. University of Natal Press, Pietermaritzburg: South Africa. p. 123.

Thomas, M.B. and A.J. Willis. 1998. Biocontrol - risky but necessary? TREE **13:** 325-329.

Uddin, W., M.D. Soika, F.E. Moorman and G. Viji. 1999. A serious outbreak of blast disease (gray leaf spot) of perennial ryegrass in golf course fairways in Pennsylvania. Plant Disease **83**: 783.

Whitehead, E.N.C. and I.S. Dunn. 1991. Economics of pastures. In: P.E. Bartholomew (ed). Agricultural production guidelines for Natal: Pastures in KwaZulu-Natal. Co-ordinated Extension Committee of KwaZulu-Natal, Department of Agriculture and Environmental Affairs, Pietermaritzburg; South Africa.

Wolfson, M.M and N.M. Tainton. 2000. Grasses: The morphology and physiology of the major forage plants. In: N.M. Tainton (ed.). Pasture management in South Africa. University of Natal Press, Pietermaritzburg: South Africa. p. 14-34.

APPENDICES

Appendix 1

FUNGICIDES (ACTIVE INGREDIENTS AND TRADE NAMES) REGISTERED FOR USE ON GRASSES

Appendix 2

PASTURE PRODUCTION SURVEY QUESTIONNAIRE (1999/2000)

Appendix 3

TURF PRODUCTION SURVEY QUESTIONNAIRE (1999/2000)

Appendix 4

COMMON AND BOTANICAL NAMES OF GRASSES REFERRED TO FOR PASTURE AND TURF ESTABLISHMENT IN THE SURVEYS (1999/2000)

Appendix 5

HERBICIDES (ACTIVE INGREDIENTS AND TRADE NAMES) REGISTERED FOR COMMONLY OCCURRING WEEDS IN PASTURE AND TURF PRODUCTION

Appendix 6

INSECTICIDES (ACTIVE INGREDIENTS AND TRADE NAMES) FOR COMMONLY OCCURRING INSECT PESTS ON GRASSES

Appendix 1

FUNGICIDES (ACTIVE INGREDIENTS AND TRADE NAMES) REGISTERED FOR USE ON GRASSES

REGISTERED FUNGICIDES			DISEASES ASSOCIATED WITH TURF AND PASTURE GRASSES																										
Active ingredient (s)	Trade name (s)	Chemical class	Anthracnose	Blast	(Gray leaf spot)	Cercospora leaf spot	Choke disease	Downy mildew	Ergot	Fairy rings	Fusarium blight	Fusarium damping off	Fusarium pink snow mould	(Fusarium patch)	Helminthosporium	Kikuyu yellows	Nematodes	(Eelworms)	Pythium blight	Pythium damping off	Rhizoctonia blight	(Brown patch)	Rhizoctonia damping off	Rust	Ryegrass toxicity	Sclerotinia dollar spot	(Dollar spot)	Smut	Spring dead spot	Take all patch
Anilazine	Dyrene					✔									✔						✔					✔				
Basic Copper sulfate	Cuproxat, Tri-basic	Inorganic	✔			✔		✔																		✔		✔		
Benomyl	Bendazole, Benlate, Benomyl, Pilarben	Benzimidazole	✔			✔					✔	✔	✔								✔			✔		✔		✔	✔	
Captab	Captan, Kaptan, Merpan	N-trihalomethylthio	✔	✔		✔					✔	✔	✔		✔						✔			✔						
Carbendazim	Bavistin, Bendazid, Delsene, Deerosal, Knowin	Benzimidazole											✔																	
Carboxin	Vitavax	Phynylamide						✔			✔	✔	✔		✔				✔	✔	✔		✔			✔		✔		
Chloroneb	Demosan, Terraneb							✔			✔	✔							✔	✔	✔		✔			✔				
Chlorothalonil	Bravo, Bravo plus, Chloro flo, Chloronil, Chlortosip, Daconil, Fungistop, Kynothal, Myroguard	N-trihalomethylthio	✔	✔	✔			✔			✔	✔	✔		✔				✔	✔	✔		✔	✔		✔		✔		
Copper oxide		Inorganic	✔			✔		✔				✔			✔					✔			✔	✔			✔			
Cyproconazole	Atemi-S	Triazole											✔								✔						✔			
Ethoprophos	Mocap EC																✔													
Etridiazol	Koban	Triazole						✔			✔	✔	✔						✔	✔			✔							
Fenamiphos	Nemacur																✔													
Fenarimol	Rubigan	Pyrimidinyl cabinol	✔								✔	✔	✔							✔				✔		✔	✔	✔	✔	✔
Fensulfotnion																	✔													
Flusilazole	Capitan, Olymp, Nustar	Silicone triazole							✔																					
Flutolonil	Prostar									✔											✔									
Folpet/Sulphur	Folpet	N-trihalomethylthio	✔																											
Fosetyl-Al	Aliette	Alkyl phosphonate						✔											✔	✔										
Iprodione	Chipco 26019, Rovral	Dicarboximide					✔				✔		✔		✔						✔		✔			✔			✔	
Mancozeb	Dithane, Ifax, Mancozeb, Manzate 200, Pennozeb, Spacozeb, Tridex	Alkylenebis (Dithiocarbomate)	✔			✔		✔			✔	✔	✔						✔	✔	✔		✔	✔		✔				
Mancozeb/Metalaxyl	Expose-MZ, Ridomil-MZ	Alkylenebis (Dithiocarbomate)						✔											✔		✔					✔				
Maneb		Alkylenebis (Dithiocarbomate)													✔									✔		✔				
Maneb/thiophanate	Coform	Alkylenebis (Dithiocarbomate)																												
Maneb/Zinc oxide	Trimangol	Alkylenebis (Dithiocarbomate)																												
Metalaxyl	Apron, Emerald, Ridomil, Subdue	Phenylamide						✔				✔							✔	✔	✔		✔			✔				
Metham	Vapam																													
Mercury Chloride													✔																	
Methyl Bromide	Methyl Bromide									✔																				
Myclobutanol																								✔						✔
Oxadixyl	Anchor	Phenylamide						✔											✔	✔										
Phenyl Mercuric Acetate																														
PCNB (Pentachloronitrobenzene)	Turfcide											✔	✔		✔					✔	✔		✔			✔				
Propamocarb hycrochloride	Banol, Previcur	Carbonate						✔				✔			✔				✔	✔			✔	✔						
Propiconazole	Banner, Novel, Practis, Tilt	Triazole	✔	✔					✔				✔		✔						✔			✔		✔		✔	✔	✔
Quintozene	Bactozene, Kynocol, PCNB, Terraclor	Aromatic hydrocarbon derivative																												
Tebuconazole	Folicur, Raxil	Triazole	✔						✔															✔		✔				
Thiabendazole	Tecto Flowable FC, Tecto 1000	Benzimidazole											✔																	
Thiophanate	Cleary 3336	Benzimidazole																												
Thiophanate-methyl	Fungo, Topsin flo, Topsin-M	Benzimidazole precursor	✔			✔					✔	✔	✔								✔		✔			✔		✔		
Thiram	Thiram, Thiulin,TMTD	Dimethyldithiocarbonate										✔	✔		✔					✔	✔		✔	✔		✔		✔		
Triadimefon	Bayleton, Bounce turfgrass fungicide	Triazole	✔								✔	✔	✔				✔				✔			✔		✔		✔		✔
Vinclosolin	Ronilan	Dicarboximide					✔						✔																✔	
Zineb	Zineb, Maneb																													

REFERENCES

Couch, H.B. 1995. Diseases of turfgrasses, 3rd edition. Krieger Publishing, Florida. p. 33-36, 59-69,111-113,136-140, 181-186, 194-197.

Krause, M.; A. Nel and K. van Zyl. 1996. A guide to the use of pesticides and fungicides in the republic of south Africa. National Department of Agriculture, Pretoria. p. 126-142; 241-279.

Nel, A.; M. Krause; N. Ramautar and K. van Zyl. 1999. A guide for the control of plant diseases. National Department of Agriculture, Pretoria. p. 54-58; 88-100;106-107.

Page, B.G. and W.T. Thomsom. 1994. The insecticide, herbicide and fungicide quick guide. Thomson Publications, California. p. 123-150.

Appendix 2

PASTURE PRODUCTION SURVEY QUESTIONNAIRE

SECTION A (personal information):

Name: .....

Manager: .....

Address:

.....

.....

.....

.....

Telephone number: .....

Fax: .....

E-mail: .....

Describe location (i.e. inland/coastal (north/south) etc):

.....

.....

SECTION B (management practices):

1. Name soil types (tick appropriate box(es)):

heavy clay ☐	sandy ☐	thin on shale ☐
humic/loamy ☐	deep black ☐	deep reds ☐

other .....

2. What pasture grasses do you use (put the approximate % of your pastures into the boxes provided)?

Eg: If you use a mixed pasture: Kikuyu [60] Cynodon [40]

Nile grass ☐	Rhodes grass ☐	Couch ☐
Star grass ☐	Desmodium ☐	Smuts finger grass ☐
Crab finger grass ☐	Teff ☐	Weeping love grass ☐
Tall fescue ☐	Annual ryegrass ☐	Perennial ryegrass ☐
Lucerne ☐	Guinea grass ☐	Dallis grass ☐
Bahia grass ☐	Kikuyu ☐	Common bristle grass ☐
Red clover ☐	White clover ☐	Garden bristle grass ☐
Rescue grass ☐	Napier grass ☐	Cocksfoot ☐
Oats ☐		

others:.....

.....

3. Irrigation (tick appropriate box(es)):

Types: center point/pivot ☐ overhead sprinkler ☐ dragline ☐

Have you consulted an irrigation engineer? yes ☐ no ☐

Do you determine the amount of water based on:

(a) the depth of the roots of the pasture? yes ☐ no ☐
(b) the water-holding capacity of the soil? yes ☐ no ☐
(c) the land slope? yes ☐ no ☐
(d) the expected evaporation losses? yes ☐ no ☐
(e) the effectiveness of your irrigation system? yes ☐ no ☐

4. Fertilization and Liming(tick appropriate box):

Is your fertilization and liming based on prior soil analysis? yes ☐ no ☐

Limes used: calcitic lime ☐ dolomitic lime ☐ gypsum ☐

Phosphate used: superphosphate ☐ DAP ☐ MAP ☐

Potassium: potassium chloride (KCl) ☐

Nitrogen: ammonium sulphate ☐ Urea ☐ LAN ☐

Mixes: 2:3:2 ☐ 2:3:2 zinc ☐ 2:3:5 ☐

Do you use a split dressing? ☐ **or do you apply 1 application yearly?** ☐

5. Grazing (tick appropriate box):

What type of a grazing program do you use?

continuous ☐ rotational - wagon wheel ☐
- blocks ☐
- strips ☐

Do you:

(a) graze 2 different classes of animals on a pasture? yes ☐ no ☐

(b) allow perennial pastures to grow out to the flowering stage at least once a year? yes ☐ no ☐

(c) use a loaf camp in your rotational program? yes ☐ no ☐

(d) apply "cleanup" cuts with a mower

- to remove uneaten stemmy material after grazing? yes ☐ no ☐
- for haymaking when growth is in excess? yes ☐ no ☐

6. **Weed problems (tick appropriate box):**

Weed types encounted:	**severe**	**occasional**	**minor**
annual grasses	☐	☐	☐
broadleaf weeds	☐	☐	☐
nutgrass	☐	☐	☐
couch	☐	☐	☐
kikuyu	☐	☐	☐

Control methods:

Chemical control ?

pre-emergent ☐ post-emergent ☐ pre- and post emergent ☐

Hormoban (Dicamba MCPA) ☐
Shell Weedkiller ☐
Pestex ☐
Tropotex ☐
Garlon ☐

2,4-D selective weedkiller ☐
Hormotox ☐
Tordon ☐
Farmers MCPA ☐

Cultural practices?

Mowing ☐ Grazing ☐ Physical labour ☐

SECTION C (pest and disease control):

1. **Insect Problems:**	**severe**	**occasional**	**minor**
Aphids	☐	☐	☐
Army worm	☐	☐	☐
Black sand mite	☐	☐	☐
Bollworm	☐	☐	☐
Birds	☐	☐	☐
Caterpillars	☐	☐	☐
Clover beetles	☐	☐	☐
Crickets	☐	☐	☐
Grasshoppers	☐	☐	☐
Ground weevils	☐	☐	☐
Ladybirds	☐	☐	☐
Locusts	☐	☐	☐
Lucerne earth flea	☐	☐	☐
Nematodes/Eelworms	☐	☐	☐
Snails	☐	☐	☐

Termites ☐ ☐ ☐
Rodents ☐ ☐ ☐
others:
.....

Control measures?

Chemical control?

Amdro	☐	Asana	☐	Astro	☐
Baytex	☐	*Bacillus thuringensis*	☐	Condor	☐
Cutlass	☐	Dibrom	☐	Dimilin	☐
Logic	☐	Malathion	☐	Methaldehyde	☐
Methomyl	☐	Methoxychlor	☐	Methyl parathion	☐
Orthene	☐	Permethrin	☐	Pyrellin	☐
Sevin	☐	Telone	☐	Thiodan	☐

others:.....
.....
Cultural practices? no ☐ yes ☐ If yes explain.....
.....
Biological control? no ☐ yes ☐ If yes explain.....
.....

2. What disease and disorders are present or have been encountered (tick appropriate boxes)?

	severe	**occas.**	**minor**	**grass(es) affected**
Blast	☐	☐	☐	.....
Choke disease	☐	☐	☐	.....
Ergot	☐	☐	☐	.....
Kikuyu yellows	☐	☐	☐	.....
Leaf blight	☐	☐	☐	.....
Rust	☐	☐	☐	.....
Ryegrass toxicity	☐	☐	☐	.....
Seedling death/damping off	☐	☐	☐	.....
Tar spot	☐	☐	☐	.....

others:
.....

Did a professional diagnose your diseases or disorders? yes ☐ no ☐

What control / curative measures did you apply ?

Chemical control: (please tick the appropriate box(es))

Anchor	☐	Apron	☐	Bayleton	☐
Benlate	☐	Bravo	☐	Bounce	☐
Punch	☐	Ridomil	☐	Sulphur	☐
Tilt	☐				

others:

.....

.....

Cultural practices? no ☐ yes ☐ If yes explain.....

.....

Biological control? no ☐ yes ☐ If yes explain.....

.....

An integrated approach? no ☐ yes ☐ If yes explain.....

.....

3. **What is your understanding of Biocontrol agents?**

.....

.....

.....

.....

.....

.....

4. **Based on you knowledge of Biocontrol agents, would you consider using one?** yes ☐ no ☐

Appendix 3

TURF PRODUCTION SURVEY QUESTIONNAIRE

SECTION A (personal information):

Name: .....

Manager: .....

Greenkeeper: .....

Address:

.....

.....

.....

.....

Telephone number: .....

Fax: .....

E-mail: .....

Describe location (i.e. inland/coastal (north/south) etc):

.....

.....

SECTION B (management practices):

1. **Percentage soil types (Place percentage into the appropriate boxes)**

sand ☐ silt ☐ clay ☐

Which of the following do you apply as a topdressing to improve soil structure

ash	☐	bark	☐	colloidal phosphate	☐
green manure	☐	animal manure	☐	peat	☐
perlite	☐	sawdust	☐	shale	☐
sludge	☐	steralised sand	☐	unprocessed sludge	☐
vermiculite	☐				

How often do you topdress?.....

To what depth do you topdress?.....

2. Greens base (tick appropriate box(es)):

USGA spec (sand based) ☐ local spec (soil based) ☐

other:.....

3. Grasses used (tick appropriate box(es)):

3.1. greens:

		cultivar name			cultivar name
Bentgrass	☐	.....	Perennial ryegrass	☐	.....
Bluegrass	☐	.....	*Cynodon dactylon*	☐	.....
C. transvaalensis	☐	.....	Buffalo grass	☐	.....
Lm grass	☐	.....	Country club	☐	.....

others:.....

.....

3.2. tees:

Kikuyu ☐ ..... *Cynodon* sp. ☐ .....

others:.....

.....

3.3. fairways:

Kikuyu ☐ ..... *Cynodon* sp. ☐ .....

others:.....

.....

4. Irrigation:

Which do you use fixed irrigation on (tick appropriate box(es)):

greens ☐ tees ☐ fairways ☐

Irrigation scheduling:

electronic ☐ tensiometer ☐ evaporation pan ☐

others:.....

.....

Time of irrigation:

early morning ☐ midday ☐ late afternoon ☐ night ☐

Irrigation equipment:

big guns (stationary)	☐	big guns (traveling)	☐
draglines and pipes	☐	pop-ups	☐
boom sprayers	☐		

others:.....

.....

Drainage systems used:

silt drainage system ☐ sand/soil drainage system ☐

all sand drainage systems ☐

5. Fertilization and Liming:

Is your fertilization and liming based on prior soil analysis? yes ☐ no ☐

Fertilizers used:

do you use a granular? ☐ or a liguid fertilizer? ☐

Fertilizer application methods:

spandicar ☐ drop-type hopper ☐

hand broadcasting ☐ foliar fertilization ☐

fertigation (granular fertilizers) ☐ fertigation (liquid fertilizers ☐

6. Mowing practices:

How often do you mow

the greens?.....

the tees?.....

the fairways?.....

What type of mower do you use

the greens?.....

the tees?.....

the fairways?.....

To what height do you mow

the greens?.....

the tees?.....

the fairways?.....

7. Decompaction and aeration

methods used to decrease compaction and increase aeration:

coring ☐ hollow tining ☐

drilling ☐ solid tine spiking ☐

8. Weed problems (tick appropriate box):

Weed types encounted:	**severe**	**occasional**	**minor**
blackjack	☐	☐	☐
castor bean plant	☐	☐	☐
various clovers	☐	☐	☐

morning glory	☐	☐	☐
milkweed	☐	☐	☐
thistle	☐	☐	☐
watergrass	☐	☐	☐
wild bindweed	☐	☐	☐
wild poinsettia	☐	☐	☐
grass invaders	☐	☐	☐

others:.....
.....

Control methods:

chemical fumigants:

Basamid	☐	Busan 1020	☐
Herbifume	☐	Methyl Bromide	☐

Herbicides:

Atrazine	☐	Basagran	☐	Cycloxydim	☐
2,4-D	☐	Dicamba	☐	EPTC	☐
Eptam	☐	Fusilade	☐	Garlon	☐
Gesaprim	☐	Gramoxone	☐	Hormoban	☐
MCPA	☐	MSMA	☐	Round-up	☐
Scatter weedkill	☐	Sting	☐	Turfweeder	☐

Cultural practices?

Mowing	☐	Physical labour	☐

SECTION C (pest and disease control):

1.	**Insect Problems:**	**severe**	**occasional**	**minor**	**products used**
	Aphids	☐	☐	☐	.....
	Army worm	☐	☐	☐	.....
	Black maize beetle	☐	☐	☐	.....
	Burrowing caterpillars	☐	☐	☐	.....
	Cutworms	☐	☐	☐	.....
	Lawn caterpillars	☐	☐	☐	.....
	Grass bag worms	☐	☐	☐	.....
	Grubs	☐	☐	☐	.....
	Leaf hoppers	☐	☐	☐	.....
	Mites	☐	☐	☐	.....
	Mole crickets	☐	☐	☐	.....
	Nematodes	☐	☐	☐	.....

Scale insects	☐	☐	☐	.....
Sod web worms	☐	☐	☐	.....
Termites	☐	☐	☐	.....

others:

.....

Cultural practices? no ☐ yes ☐ If yes explain.....

.....

Biological control? no ☐ yes ☐ If yes explain.....

.....

3. What disease and disorders are present or have been encountered (tick appropriate boxes)?

	severe	**occas.**	**minor**	**grass(es) affected**
Anthracnose	☐	☐	☐	.....
Brown patch	☐	☐	☐	.....
Brown spot	☐	☐	☐	.....
Cynodon decline	☐	☐	☐	.....
Cynodon leaf spot	☐	☐	☐	.....
Dollar spot	☐	☐	☐	.....
Downy mildew	☐	☐	☐	.....
Fairy rings	☐	☐	☐	.....
Kikuyu leaf spot	☐	☐	☐	.....
Kikuyu yellow	☐	☐	☐	.....
Fusarium patch	☐	☐	☐	.....
Pythium blight	☐	☐	☐	.....
Spring dead spot	☐	☐	☐	.....
Take-all patch	☐	☐	☐	.....
Turfgrass rust	☐	☐	☐	.....

others:

.....

What control / curative measures did you apply ?

Chemical control:		diseases controlled
Alliette	☐	.....
Anchor	☐	.....
Apron	☐	.....
Bayleton	☐	.....
Benlate	☐	.....
Bounce	☐	.....
Bravo	☐	.....
Folicur	☐	.....

Punch ☐ .....
Previcur ☐ .....
Ridomil ☐ .....
Rovral ☐ .....
Sulphur ☐ .....
Tilt ☐ .....
others:
.....

Cultural practices? no ☐ yes ☐
Reduced organic matter ☐
Nitrogen manipulation ☐
Improved drainage ☐
Irrigation management ☐
Soil structure improvement ☐
Reduced stress ☐
Topdressing ☐
others:
.....

Biological control? no ☐ yes ☐ If yes explain.....
.....
An integrated approach? no ☐ yes ☐ If yes explain.....
.....

3. What is your understanding of Biocontrol agents?

.....
.....
.....
.....
.....
.....

4. Based on you knowledge of Biocontrol agents, would you consider using one? ☐ yes ☐ no

If yes explain.....
.....
.....

Appendix 4

COMMON AND BOTANICAL NAMES OF GRASSES REFERRED TO FOR PASTURE AND TURF ESTABLISHMENT IN THE SURVEYS (1999/2000)

Common name	Botanical name
Pasture grasses referred to (See Figure 3.2)	
Annual ryegrass	*Lolium perenne*
Cocksfoot	*Dactylis glomerata*
Couch grass	*Cynodon dactylon*
Dallis grass	*Paspalum dilatatum*
Kikuyu	*Pennisetum clandestinum*
Nile grass	*Acroceras macrum*
Perennial ryegrass	*Lolium multiflorum*
Rescue grass	*Bromus catharticus*
Rhodes grass	*Chloris gayana*
Smuts finger grass	*Digitaria eriantha*
Tall fescue	*Festuca elatior*
Teff	*Eragrostis teff*
Weeping love grass	*Eragrostis curvula*
Turf grasses referred to (See Figure 3.13)	
Bayview	*Cynodon transvaalensis*
Bentgrass	*Agrostis stolonifera*
Buffalo grass (coastal)	*Stenotaphrum secundatum*
Country club	*Paspalum vaginatum*
Couch grass/ Bermuda grass	*Cynodon dactylon*
Kikuyu	*Pennistetum clandestinum*
Lm grass	*Dactyloctenium australe*

References

Gibbs Russell, G. E.; L. Watson; M. Koekermoer; L. Smook; N.P. Barker; H.M. Anderson and M.J. Dallwitz. 1990. Grasses of Southern Africa. Botanical Research Institute, South Africa.

Oudsthoorn, F. 1999. Guide to grasses of southern Africa. Britz Publishers, Pretoria: South Africa.

Appendix 5
HERBICIDES (ACTIVE INGREDIENTS AND TRADE NAMES) REGISTERED FOR COMMONLY OCCURRING WEEDS IN PASTURE AND TURF PRODUCTION

REGISTERED HERBICIDES			WEED TYPES										
			Pre-emergent	Post-emergent		Annual grasses	Perennial grasses	Annual broad-leaved weeds	Perennial broad-leaved weeds	Purple nutsedge	Yellow nutsedge	Bush encroachment, noxious weeds
Active ingredient (s)	**Trade name (s)**	Herbicide Classification										
Atrazine	Atra, Atraflo, Atrasien, Atrazol	Triazines	✔					✔				
Bendioxide	Basagran	Benzothiadiazole		✔				✔			✔	
Cycloxydim	Focus Ultra	Cyclohexanediones		✔		✔	✔					
2,4-D/dicamba/MCPA	Lawn Weedkiller, Turfweeder	Phenoxy-carboxylic acids, Benzoic acids	✔	✔				✔				
Dicamba/MCPA	Hormoban	Phenoxy-carboxylic acids, Benzoic acids		✔				✔	✔			
EPTC	Eptam, Eradicate Plus	Thiocarbamates	✔			✔				✔	✔	
Glyphosate	Roundup, Sting	Glycines	✔			✔	✔	✔	✔			✔
Halosulfuron	Servion	Sulfonylureas		✔						✔	✔	
Imazapyr	Chopper	Imidazolinones		✔		✔	✔	✔	✔		✔	✔
Methyl Bromide [a]	Methyl Bromide											
MCPA	Kombat Weeds	Phenoxy-carboxylic acids		✔				✔				
MSMA	Magma, Target MSMA, Masmar	Organoarsenicals		✔		✔		✔	✔	✔	✔	
Propyzamide	Kerb	Benzamides	✔	✔		✔						
Triclopyr	Garlon	Pyridine carboxylic acids		✔								✔

[a] withdrawn in December 1995

REFERENCES

Vermeulen, J.B., M. Deyer, H. Grobler and K. van Zyl. 1996. A guide to the use of herbicides. NAtional Department of Agriculture, Pretoria: South Africa
Grobler, H.; J.B. Vermeulen and K. van Zyl. 2000. A guide to the use of herbicides. National Department of Agriculture, Pretoria: South Africa.

Appendix 6

INSECTICIDES (ACTIVE INGREDIENTS AND TRADE NAMES) FOR COMMONLY OCCURRING INSECT PESTS ON GRASSES

REGISTERED INSECTICIDES		INSECT PESTS ASSOCIATED WITH TURF AND PASTURE GRASSES																					
		Aphids	Armyworms	Black maize beetle	Black sand mite	Bollworm	Caterpillars	Clover beetles	Crickets	Cutworms	Lucerne earth flea	Grass bag worm	Grasshoppers	Ground weevils	Grubs/larvae	Leafhoppers	Locusts	Kikyu tiporer	Nematodes	Red spider mite	Snails and slugs	Termites
Active ingredient (s)	**Trade name**																					
Bacillus thuringiensis	Cutlass					✔	✔		✔						✔							
Carbaryl	Sevin		✔				✔						✔		✔						✔	✔
Chlorpyrifos	Dursban		✔			✔	✔			✔												
Cyfluthrin	Sneak		✔	✔		✔	✔		✔	✔		✔										✔
Cypermethrin	Cypermethrin		✔			✔	✔		✔	✔		✔		✔								✔
Endosulfan	Thiodan	✔	✔			✔				✔						✔		✔				
Fenamiphos	Nemacure																		✔			
Isofenphos	Peril						✔		✔						✔							
Lamba-cyhalothrin	Karate					✔				✔								✔				
Mercaptothion	Malathion	✔	✔				✔		✔				✔		✔	✔				✔	✔	
Omethoate	Folicur	✔					✔				✔											
Permethrin	Permethrin		✔		✔	✔	✔		✔	✔	✔											✔
Phoxim	Turmoil																					✔

REFERENCES

Krause, M.; A. Nel and K. van Zyl. 1996. A guide to the use of pesticides and fungicides in the republic of south Africa. National Department of Agriculture, Pretoria. p. 126-142; 241-279.