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The role of fungi and the root lesion nematode, *Pratylenchus neglectus*, in damaging wheat roots in South Australia

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SUMMARY

Root systems of wheat (*Triticum aestivum*) suffer extensive damage, which is ubiquitous in South Australian crops. Seminal roots are subject to cortical discolouration and rotting. Crown roots display extensive decortication and orange-brown lesions, and appear as rotted "stumps" or "spikes" by the end of the growing season. Growth of lateral roots on seminal and crown root axes is inhibited. Existing studies on root pathogens in South Australia do not adequately explain the cause of such damage.

Pathogens associated with this root damage were investigated in the Murray Mallee region of South Australia over the 1987 - 1989 growing seasons. Occurrence of fungal species and the root lesion nematode (*Pratylenchus neglectus*) was assessed, and related to the appearance and severity of symptoms on the roots. Field experiments were supplemented with inoculation tests in the glasshouse and laboratory.

Fusarium spp., *Pythium* spp. and *Microdochium bolleyi* were the fungi most frequently encountered. In 1989, the first season in which the nematode was included in identifications of organisms, *P. neglectus* was invariably associated with damaged roots. Fungi considered to be "major" pathogens of wheat crowns and roots (*F. graminearum*, *Gaeumannomyces graminis* and *Rhizoctonia solani*) were isolated infrequently and were not significantly associated with root damage. Numerous other fungi were identified, but these occurred sporadically and at low frequencies, and could not be related to the severity of root damage.

Bipolaris sorokiniana, a well-characterised pathogen, infected 11% of subcrown internodes sampled in 1987, but only 2 - 3% in 1988 and 1989. The relationship between poor plant growth and the presence of this pathogen was tenuous. Disease rating was not positively correlated with the frequency of *B. sorokiniana* isolated from subcrown internodes, but was correlated with *Fusarium* spp. or with *M. bolleyi*. The fusaria infected 65 - 77% of subcrown internodes in 1987, and *M. bolleyi* colonised 8 - 20% of these subcrown internodes.

The hypothesis that *M. bolleyi* augments disease caused by *B. sorokiniana* was tested. However, under controlled conditions, *M. bolleyi* reduced infection levels attained

by *B. sorokiniana,* and plants suffered less root damage. The mechanism whereby *M. bolleyi* inhibited *B. sorokiniana* seems to be one of competition. This relationship was less obvious in the field, due to interference from biotic, climatic and edaphic factors.

M. bolleyi readily infected wheat roots, and occurred at high levels in fields examined. In 1987 - 1989, 13 - 26% of root segments sampled were infected by this species. Although this fungus was isolated at a significantly higher frequency from roots with poorly developed laterals than from those with healthy lateral roots, plants inoculated with *M. bolleyi* alone suffered negligible damage.

Fusarium spp. and *Pythium* spp. were isolated from a large proportion of roots examined. *Fusarium* spp. (other than *F. graminearum*) infected 36 - 95% of roots in 1987 - 1989, and 79% of roots were colonised by *Pythium* spp. in 1989. These fungi significantly increased in frequency as the severity of crown root damage increased. The frequency of fusaria was significantly higher in plants with lateral root damage than in those with healthy laterals.

Plants inoculated with both *P. neglectus* and *Pythium* sp. sustained more root damage than those inoculated with either pathogen alone. Their combined influence on plant growth was particularly obvious in retarding lateral root growth. Some of the symptoms observed on roots of field-grown plants were reproduced by inoculation with these organisms: decortication, "spear" root tips and inhibition of lateral root growth. However, the nematodes used to inoculate plants were not aseptic, so fungi and bacteria also may have been introduced.

P. neglectus infected all 21 cereal and eleven legume varieties tested, but there were differences between and within species in the nematode populations they supported. Of the varieties tested, Harbinger (*Medicago littoralis*) and Jemalong (*M. truncatula*) medics, and Gungurru lupin (*Lupinus angustifolius*) were infected with the least *P. neglectus* per plant.

P. neglectus alone caused little damage to wheat roots, although limited areas had been decorticated. In the field, *P. neglectus* in conjunction with at least one, but more likely several, species of fungi may be needed for full disease expression. Amethyst chickpea (*Cicer arietinum*) was readily invaded by *P. neglectus*, resulting in distinct

lesions on and within roots, and inhibition of lateral root growth. This chickpea would be an excellent species on which to study the epidemiology and aetiology of root disease caused by *P. neglectus* plus fungi, although the fungi damaging legume roots may be different from those damaging cereal roots.

The damage sustained by wheat roots is likely to result from *P. neglectus* feeding within the cortex, followed by the invasion of root rotting fungi (*Fusarium* spp. other than *F. graminearum*, *Pythium* spp., *M. bolleyi*) and bacteria. These results, combined with the surveys of others indicating that *P. neglectus* is widespread in South Australian cereal crops, suggest that the root lesion nematode initiates the lesioning and rotting observed throughout the State's wheat belt.

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This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this thesis being made available for photocopying and loan.

Vivien A. Vanstone

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CHAPTER 1

GENERAL INTRODUCTION

Despite more than a century of root disease studies in South Australia, the root systems of cereals continue to suffer extensive damage, especially on the lighter soils of the State's wheat belt. Control measures are not entirely successful, although appropriate rotations and cultural practices may reduce the severity of disease. This has been demonstrated on the property of D. M. Correll on the Yorke Peninsula of the State, where the yield of wheat and barley breeding trials has increased from approximately 1.0 tonne/ha in the 1960's to 3.0 - 4.0 tonnes/ha in the 1980's.

The aim of the present study was to identify those organisms associated with the widespread occurrence of generalised rotting and damage to wheat roots. Such root damage causes an overall reduction of vigor in crops, and consequently leads to diminished grain yields.

In recent years there have been, in effect, two hypotheses put forward to explain the cause of root disease and consequent yield losses. These ideas are now widespread in the farming and scientific communities. That favoured by Dr A. D. Rovira and his co-workers, CSIRO (Commonwealth Scientific and Industrial Research Organisation) Division of Soils, is that yield loss is attributable to the sub-clinical effects of *Gaeumannomyces graminis* var. *tritici* ("take-all"). Yield reduction in wheat due to this sub-clinical disease has been estimated at approximately 10% (Stynes, 1975; Rovira, 1978). However, despite much research, there are still no reliable quantitative data proving the economic significance of *G. graminis* (Patel, 1983; Wallace, 1987). Detailed isolation and identification of root-inhabiting fungi by J. R. Harris and R. Moen (CSIRO Division of Soils), Dr H. Wallwork and Dr P. A. Pittaway (Waite Institute) and in this thesis has failed to demonstrate sub-clinical expression of the disease. Although *G. graminis* may infect as many as 50% of the seedlings in a crop, the occasional lesion on a root system is of no practical significance, and is no indication of a potential yield reduction. The fungus is of importance only when it invades the crown, the tiller bases,

the majority of seminal roots near the seed, or all three. Such severe infections can then be discerned as irregular patches of poor growth in the crop. This is particularly evident in seasons where a wet winter-spring is followed by a period of water stress.

The alternative hypothesis appears even more unlikely. During the past decade, and especially in the last five years, it has become common to ascribe root lesions, and by implication root damage and yield loss, to the fungus *Rhizoctonia solani*. Kerr (1955), however, demonstrated that although 70% of *R. solani* isolates from within "bare patches" were pathogenic to wheat, only 12.5% of those from outside patches were virulent. Formation of patches, and thus significant yield loss, occurs only when high populations of pathogenic *R. solani* strains are present (Kerr, 1955; de Beer, 1965). To ascribe root damage to *R. solani* requires that the fungus is present within root tissues and that it is pathogenic. These criteria have not always been met in recent studies, and there is no published evidence disputing the findings of Kerr (1955) and de Beer (1965).

Like *G. graminis*, *R. solani* appears to be encountered rarely in crops. Wallace (1987) maintains that data are not sufficient to determine the economic importance of *R. solani*. Furthermore, *R. solani* is usually only associated with serious crop losses on the white, sandy soils south-east of the River Murray and on some areas of the Eyre Peninsula. Stynes (1975) associated *R. solani* with a 1.6% yield loss on the Yorke Peninsula, and Banyer (1965) reported only slight damage due to *R. solani* in wheat crops in the Lower North of South Australia.

Many wheat crops grown on the light, sandy soils of the Murray Mallee show a general lack of vigor throughout the growing season, with poor stands and reduced tillering. It is only infrequently that distinct "patches" are encountered, but small areas of less vigorous growth (less than 3.0 m in diameter) are often observed throughout the crop. Plant growth and heading are uneven across the paddock.

Seminal roots are subject to cortical discolouration and occasional rotting. Distinct lesions, either stelar or cortical, are not always evident on the seminal root system. The crown roots suffer extensive damage, comprising rotting, decortication and numerous orange-brown lesions. By the end of the season, most crown roots have been reduced to short, rotted "stumps" or "spikes". Crown root damage must severely restrict plant

growth and yield, especially in areas of low water and nutrient availability. Rotting of crown roots is particularly damaging if the function of the subcrown internode and seminal roots has already been impaired due to attack by pathogens. During the course of this study, it became evident that lateral roots suffered considerable damage. Lateral roots along both seminal and crown root axes are stunted and rotted. Many areas of the root system are devoid of laterals, further restricting root function. Growth of root hairs is also inhibited when root axes and laterals are damaged.

These observations lead to the postulation of two explanations. Either an unrecognised organism (or organisms) is primarily responsible for the root damage, or the symptoms observed in the field are due to the combined effects of several root-attacking organisms. The latter explanation is favoured by J. R. Harris and R. Moen, whose extensive isolations from rotted cereal roots in South Australia have revealed a plethora of fungi within these tissues. Roots are naturally exposed to a vast fungal flora. Warcup (1957) detected over 210 species in a wheat field soil at the Waite Institute.

The root lesion nematode (*Pratylenchus neglectus*, syn. *P. minyus*) has been recognised in South Australia since 1956 (J. M. Fisher, personal communication), but its role in cereal root disease has never been clearly defined. However, this nematode is often associated with diseased cereal roots in South Australia (de Beer, 1965; Kimpinski, 1972; Stynes, 1975; Stynes and Veitch, 1983; Patel, 1983). As the work towards this thesis progressed, more attention was focused on the role of this nematode. *P. neglectus*, alone or in conjunction with fungi, may be responsible for the observed root damage.

Microdochium bolleyi is generally considered to be a "minor" pathogen, but is often isolated from diseased cereal roots in South Australia (Harris, 1986), where it may be involved in a root disease complex, along with *Bipolaris sorokiniana* and *Fusarium* spp. *M. bolleyi* seems to have escaped serious study as a cereal root pathogen. The pathogenicity of *B. sorokiniana* is well established, but not all *Fusarium* spp. are considered damaging to cereal roots. However, R. Moen (personal communication) considers that any fusaria isolated from roots should be considered as potentially damaging. Among the many fungi isolated from diseased cereal roots in South Australia, *Fusarium* spp. constitute a large proportion of the isolates (Moen and Harris, 1980;

Fedel-Moen and Harris, 1987).

Many species of *Pythium* are isolated from cereal roots in South Australia (Bratoloveanu, 1985; Pankhurst and McDonald, 1988b), where these are considered to reduce barley yields (Bratoloveanu and Wallace, 1985). Pittaway and Rathjen (1984) frequently detected *P. graminicola* in wheat roots. They considered it a potentially important pathogen in causing root disease in conjunction with other fungi, although Mayfield (1981) reported that losses due to *Pythium* spp. in South Australia were slight. However, in the United States, Cook *et al.* (1980) were able to increase wheat yields by 40% when using a fungicide selective for *Pythium* spp.

In the field, roots are not infected by only one organism. Plants showing symptoms of "major" diseases like "take-all" or *Rhizoctonia* root rot are also colonised by numerous other species. Sturz and Bernier (1987a) detected *B. sorokiniana*, *M. bolleyi* and six species of *Fusarium* in the roots of wheat displaying symptoms of "take-all". Plants with root damage that, in South Australia, is usually attributed to *R. solani* are also infected with other fungi, such as those described in Western Australia by Roberts and Sivasithamparam (1986, 1987). Identification of all organisms present is thus critical in disease diagnosis. Plants must be removed from the soil, the roots examined and organisms identified, especially as above-ground symptoms are often non-specific or lacking.

These associated organisms cannot be ignored in the development of disease. The work of Harris and Moen (1985a, 1985b) and Moen and Harris (1985) in South Australia agrees with this observation. The number of fungi inhabiting root tissues far exceeds the number that cause the conventional "major" diseases (Salt, 1979). Symptoms alone are thus a poor indication of the identity of organisms present, and so-called "minor" pathogens may significantly contribute to the degree of root damage encountered. However, the majority of research focuses on the study of a single pathogen, with various workers tending to investigate a particular disease which appears to be caused by a particular organism. Under field conditions, this just does not happen, because roots are invaded by a complex array of microorganisms.

Since isolates of "minor" pathogens are largely ignored, this could result in a

failure to recognise potentially important aetiological complexes (Hill *et al.*, 1983). These fungi can become quite damaging under conditions of host plant stress (Harris, 1987), especially if roots are already colonised by other organisms. Many of these species have not been examined adequately and, consequently, their role in root disease is not well understood. Even though "major" diseases are absent, maximum yield potential is often not achieved (Salt, 1977).

As the relative frequencies of these organisms tend to change during the plant's life, sampling must be carried out over the entire growing season. It is difficult to define the causal agents of root disease, and relate the presence of root rotting organisms to the level of damage observed in the field. Furthermore, evaluations of the importance of organisms in the field rarely coincide with the results obtained from inoculation experiments under controlled conditions. Environmental factors, both biotic and abiotic, modify the influence these organisms have on plants, as well as modifying the interactions that occur between soil-borne organisms.

This thesis reports an attempt to determine the organisms involved with inhibition and rotting of lateral roots, decortication of roots, and the destruction of crown root systems. The occurrence of pathogens was investigated under the conditions encountered in the Murray Mallee region of South Australia, where average rainfall is low and sandy surface soils of low inherent fertility predominate. Some of the interactions that occur between root rotting organisms were also investigated. Study of the interrelationships between pathogens is crucial in understanding the effect of various organisms on roots, and it is imperative before breeding programs can be implemented to incorporate genetic mechanisms of limiting the damage to wheat root systems.

CHAPTER 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

Although fungi are the most common incitants of wheat diseases in Australia, with all the major fungal groups represented (Murray and Brown, 1987), wheat roots are also attacked by several species of nematode. In South Australia, the cereal cyst nematode (*Heterodera avenae*) is damaging and widespread. However, the use of appropriate crop rotations, combined with resistant cereal cultivars and improved crop nutrition, has considerably minimised losses caused by this nematode (Fisher and Hancock, 1991). Root lesion nematodes (*Pratylenchus neglectus* and *P. thornei*) infect the roots of many crop and pasture species, although their role in causing damage to cereal roots in South Australia has not been clearly defined. *P. neglectus* is frequently associated with damaged roots that are also colonised by fungi (Benedict and Mountain, 1956; Price, 1970; Kimpinski, 1972; Kimpinski *et al.*, 1976; Patel, 1983), and is widespread in most cereal growing districts of South Australia (de Beer, 1965; Kimpinski, 1972; Stynes, 1975; Patel, 1983).

Field studies reveal a plethora of fungi that colonise root and crown tissues. *Gaeumannomyces graminis*, *Bipolaris sorokiniana*, *Fusarium* spp., *Pythium* spp. and *Rhizoctonia solani* have been extensively studied on cereals. Studies on these organisms alone, however, do not appear to account for the damage to wheat roots in South Australia. *Microdochium bolleyi* is also extremely common in cultivated soils, but has gained little attention as a pathogen of wheat. After defining the structure of cereal root systems, the potential contribution of these organisms to root damage will be reviewed, and the interrelationships that occur between them will be examined.

2.2 STRUCTURE AND FUNCTION OF CEREAL ROOTS

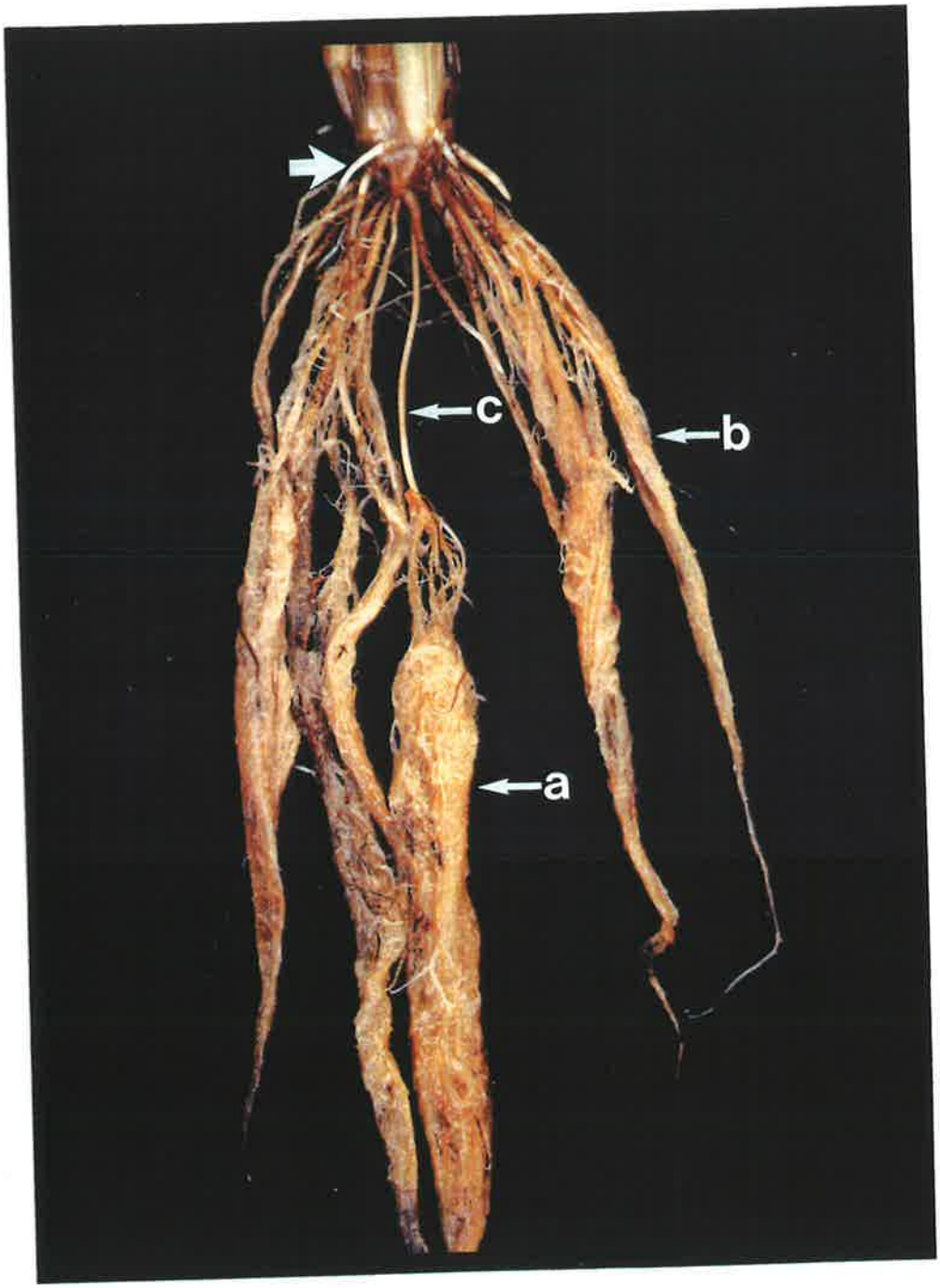
The wheat root system consists of seminal and crown roots both of which, in the healthy plant, produce lateral root branches and root hairs along the entire zone of cell differentiation. Some aspects of the structure and function of these roots will be reviewed, and the terms used to refer to these structures will be defined. The morphology of the wheat root system is demonstrated in Plate 2.1.

Seminal roots (also known as primary roots) develop from primordia in the embryo, and wheat plants produce three to six of these root axes (Weaver, 1926; Pavlychenko, 1937; Manner, 1957), plus three from the coleoptile node. There is some disagreement regarding the importance of these roots to the plant. According to Nelson (1946), wheat seminal roots are only temporary structures to supply the needs of the plant at the seedling stage, and Sallans (1942) thought that these roots were only important until about half way through the growing season. However, seminal roots function throughout the life of the plant (Hector, 1936), with extension continuing until plant maturity (Walter, 1971). In fact, plants can grow on seminal roots alone (Weaver and Zink, 1945), although injury to or restriction of either seminal or crown roots can lead to enhanced activity of the other (Russell, 1977). Seminal roots are essential for water and nutrient extraction at depth, while crown roots can take advantage of light rain showers and nutrients in the cultivated layer of the soil. In fact, cereal crops are supported exclusively by the seminal roots under very dry conditions when crown roots fail to develop (Pavlychenko, 1937). In the field, variation in water supply is often the major factor determining root distribution (Russell, 1977) within the soil profile.

Crown roots (also known as adventitious, coronal, nodal or secondary roots) arise adventitiously from the main stem and tillers of the plant, and (as soon as they appear) these roots are able to absorb nutrients (Williams, 1962). Once initiated, crown roots are produced continuously throughout the life of the plant (Schuurman and de Boer, 1970), although their rate of growth is affected by climatic and edaphic factors, including attack by soil-borne pathogens. The number of crown roots varies widely. Oat plants, grown singly in containers, may produce as many as 184 crown roots (Schuurman and

PLATE 2.1: The root system of wheat consists of seminal roots (**a**), crown roots (**b**) and a subcrown internode (**c**). Seminal and crown roots are "matted" due to the presence of numerous lateral root branches. Under field conditions, crown roots are initiated when plants are about six weeks old. These roots are then produced continuously throughout the life of the plant, and newly developing crown roots can be seen emerging from the crown (➡).

Plate 2.1



de Boer, 1970), but barley grown in solution culture only produced nineteen crown roots (Hackett, 1968). Under field conditions, Pavlychenko (1937) recorded eleven crown roots on mature Marquis wheat plants.

The ability of a root system to become established at depth is one of its most important characteristics (Russell, 1977). Weaver (1926) found that, in some environments, wheat roots were abundant at depths of over 100 cm, with some reaching below 200 cm. Pavlychenko (1937) recorded similar figures: seminal roots penetrated to a depth of 115 cm, and crown roots to 71 cm. The maximum length of oat seminal roots recorded by Schuurman and de Boer (1970) was 70 cm. However, in the field, roots only explore a small volume of the soil available, and many die before crop maturity (Russell, 1977). Lungley (1973) calculated that cereal roots spread approximately 20 cm from the plant's crown, with 20% of the root mass occurring in the top 10 cm of soil. Walter (1971), however, observed that 50% of wheat roots were present in this region.

Some phenomenal measurements of root growth have been made, indicating the plant's potential to grow under optimal conditions. Dittmer (1937) assessed root growth of a single, container-grown rye plant at four months of age. The entire root system contained nearly fourteen million members, with a total length of 623 km, covering a surface area of 650 m^2. The root hairs constituted more than two thirds of this surface area. The subterranean surface area of this plant was 130 times that of the total exposed shoot area, or 22 times if the internal transpirational surfaces of leaves were considered. Pavlychenko (1937) examined the root system of field-grown Marquis wheat. The seminal roots plus their lateral branches had a total length of 210 m, and crown roots plus branches measured 49 m.

Lateral roots are formed some distance behind the main root apex, and this distance, under standard growing conditions, is constant for each species (McCully, 1975). These roots are formed endogenously, in acropetal sequence, and must grow through the cortex and epidermis of the parent root. Primary laterals are produced, which in turn can give rise to secondary laterals. Many factors influence the branching pattern of roots, but the location of laterals is related to the vascular pattern of the parent root, and their dispersal along the root axis is non-random (McCully, 1975). If roots are curved for

any reason, laterals will develop on the convex side of the main root axis. Weaver (1926) remarked that lateral roots under field conditions grow "over half an inch per day" (1.3 cm), whereas under laboratory conditions Lungley (1973) measured growth rates of only 0.5 cm per day for these roots. Growth rate will vary considerably, depending on numerous environmental factors.

Lateral roots make up a considerable portion of the root system. Although their individual volumes are less than that of main axes, laterals have a much greater total length and surface area (Russell and Clarkson, 1976). Lungley (1973) calculated that primary laterals accounted for 80% of the total root length of field-grown small grain cereals 60 days of age, and secondary laterals comprised most of the remainder. Schuurman and de Boer (1970) measured individual primary laterals of oat plants up to a length of 52 cm. Barley grown in solution culture, with ample nutrients, can produce 2000 laterals with a total length of almost 50 m, whereas the main seminal and crown root axes combined only accounted for 8 m of the root system (Hackett, 1968). Pavlychenko (1937) counted 1873 primary laterals on seminal roots and 912 on crown roots of mature Marquis wheat roots under field conditions. These roots had a total length of 251 m, while seminal and crown roots together measured only 8 m.

Lateral roots make a vital contribution to the uptake of water and nutrients, and Pavlychenko (1937) went as far as to say that the fine rootlets represented the active absorptive surfaces of the whole root system. Russell and Sanderson (1967) estimated that the laterals of barley were responsible for 55% of phosphate uptake, while crown and seminal roots, respectively, accounted for only 30% and 15% of the uptake. However, crown roots actually translocated more absorbed phosphate ions to the shoots than did either seminal or lateral roots. Russell and Sanderson (1967) concluded that differences in root volume accounted for some of the discrepancies between absorption and translocation capacities of the three types of root, but this was not the sole reason for these discrepancies. If one part of the root system develops in a more favourable zone in the soil, a localised proliferation of lateral roots can occur (Russell, 1977). This is a feature of root growth commonly observed with the application of nitrogenous fertilisers.

Seminal, crown and lateral roots also react differently to nutrient deficiencies.

Hackett (1968) found that nutrient deficiencies in barley grown in solution culture retarded crown root growth more than that of seminal roots. Potassium or phosphorus deficiencies reduced the number of lateral root axes produced and inhibited the growth of primary laterals, while potassium deficiency completely inhibited the growth of secondary laterals.

A subcrown internode, which is, morphologically and anatomically, a stem-like connection, forms between the coleoptile node and the first foliar node (Plate 2.1). The length of this internode depends on seeding depth and plant genotype. Some pathogens, like *Bipolaris sorokiniana*, preferentially attack this part of the root system (Fedel-Moen and Harris, 1987). Overall, however, the subcrown internode is less vulnerable to infection by microorganisms than are the roots (especially the root hairs and thinner laterals).

Root hairs originate as protuberances from epidermal cells (Russell, 1977) in the zone of cell differentiation. Environmental factors influence root hair growth, but these roots typically develop in profusion over the entire root in this zone. Root hairs are particularly vulnerable to microbial attack, and usually survive only a few days (Russell, 1977). However, Dittmer (1937) calculated that a rye plant could produce over 118 million new root hairs per day. These roots, with a total surface area nearly twice that of all other roots combined (Dittmer, 1937), absorb water and nutrients, but are not essential for either purpose (Russell, 1977). Root hairs also anchor young roots as they grow through the soil.

Seminal and crown roots differ in their internal structure but, physiologically, perform similar functions. Mature roots of monocotyledons are a cylinder of approximately uniform diameter, except when environmental changes modify the size of the meristem, or outer tissues collapse due to age or microbial attack (Russell, 1977). The epidermis, one cell thick, is the outermost layer of root cells. A root hair and the epidermal cell from which it arises constitutes a single cell. The cortex is the inner layer of cells surrounding the vascular tissue (or stele). Cell walls and intercellular spaces of the cortex freely imbibe water and solutes from the surrounding soil. An endodermis, one cell thick, encircles the stele. Endodermal cells exercise some control over the passage of

water and solutes between cortex and stele. Vascular tissue is comprised of xylem which conveys water and solutes to the shoot, and phloem which transports metabolites downwards to the roots.

Roots are, of course, greatly influenced by the activities of soil-borne pathogens. However, some root decortication is common, even when pathogens are absent, but this has no apparent effect on root growth, unless it is particularly severe (Greaves and Darbyshire, 1975), or occurs at an early stage of root growth prior to lateral root formation. Holden (1975, 1976) also noted that roots are subject to some degree of natural cortical senescence. If roots suffer any serious damage at emergence, plant death invariably results (Pavlychenko, 1937). Soil-borne organisms thus exert considerable influence on root growth and, in turn, root growth influences the activities of pathogens and other soil inhabitants in the rhizosphere.

2.3 *PRATYLENCHUS* SPP.

Species of the nematode *Pratylenchus* Filipjev 1936 (Order: Tylenchida) are migratory endoparasites that feed on a wide range of cultivated and wild hosts in most regions of the world (Wiese, 1987). These nematodes frequently predispose plants to attack by other soil-borne pathogens, producing conspicuous lesions (Thorne, 1961), which prompted Godfrey (1929) to describe *Pratylenchus* spp. as "root lesion nematodes".

P. thornei Sher and Allen 1953 and *P. neglectus* (Rensch 1924) Chitwood and Oteifa 1952 (syn. *P. minyus* Sher and Allen 1953) attack wheat and other cereals, the former having been more thoroughly studied on cereal hosts than the latter. *P. neglectus* is primarily a parasite of grasses, but infects a range of other crops, including legumes, crucifers, flowers, strawberry, fruit trees (Goodey *et al.*, 1965; Tobar Jimenez, 1971), tobacco (Mountain, 1954), peppermint (Faulkner and Skotland, 1965; Faulkner and Bolander, 1969), red clover, soybean (Mountain, 1954), vetch, chickpea (Guevara-Benitez *et al.*, 1970) and white clover (Townshend and Potter, 1976).

Life Cycle

Males of *P. neglectus* are of no reproductive or diagnostic significance, and only three male specimens have been identified (Townshend and Anderson, 1976). Females reproduce by mitotic parthenogenesis. Individuals are between 0.31 mm and 0.55 mm in length (Thorne, 1961), although there is considerable variation in body length, width and tail shape (Loof, 1960). Larvae go through four moults, the first within the egg. Adults and larvae are motile within the soil and host tissues (Wiese, 1987). Both juveniles and adults penetrate roots, moving through the cortex, depositing eggs as they feed.

The life-cycle of *P. neglectus* may be completed on tobacco within 28 days (Mountain, 1954), and Baxter and Blake (1968) observed second stage *P. thornei* larvae 24 days after wheat had been inoculated with adults. However, the life cycle of *P. neglectus* has not been studied under South Australian conditions, and may be quite different from that of *P. thornei* or *P. neglectus* studied under other agricultural systems and environmental conditions.

Eggs and other stages become quiescent in the soil (entering an anhydrobiotic state) during periods of host unavailability or adverse climatic conditions (Wiese, 1987), but are unable to survive sub-zero temperatures (Townshend and Anderson, 1976). The moisture content of soil is a critical factor determining survival of *P. thornei* (Baxter and Blake, 1968). In South Australia, Grandison (1972) reported *P. thornei* more frequently on clover roots in clay than in sandy soils, where water availability was the most significant factor determining nematode activity in the soil. Stynes (1975) concluded that soil properties had no direct influence on *P. neglectus*, but the effect of edaphic conditions on soil water content indirectly influenced nematode populations. Kimpinski (1972) came to the same conclusion, and found that precipitation (as it affects growth of the host and nematode motility) was the most important factor influencing the abundance and activity of *P. neglectus*.

Invasion of Roots

Pratylenchus spp. are attracted to roots, particularly the tips, which is probably a chemokinetic response (Townshend and Anderson, 1976). The nematodes first feed

externally on meristematic tissue behind the root cap, causing elongation to cease (Townshend and Anderson, 1976), and eventually inhibiting growth of root tips and laterals (Corbett, 1972). *Pratylenchus* spp. rarely penetrate the meristem, but migrate to more mature areas where a site for penetration is found. *P. thornei* do not invade wheat roots randomly, but are attracted to areas where penetration has already occurred (Baxter and Blake, 1967). *P. neglectus* force epidermal cells of tobacco roots aside or penetrate these cells directly. Within four to six hours the nematodes invade cortical cells (Mountain, 1955), where they become stationary four to six days prior to migration through the root to feed on other cells. Eggs are laid almost immediately upon the initiation of feeding, hatching within seven to nine days. The eggs are laid singly or deposited in groups or linearly in the track of the feeding female (Baxter and Blake, 1968). Numbers of *P. thornei* in wheat roots thus increase exponentially with time, as do populations of other *Pratylenchus* spp. Cell walls disintegrate as the cytoplasm is withdrawn, forming cavities within the cortex (Baxter and Blake, 1968). Cavities formed by individual nematodes coalesce, the epidermis sloughs off and the exposed stele often becomes necrotic. The older, dying roots are then vacated in a search for fresh tissue (Wiese, 1987).

Symptoms

Young roots become discoloured and stunted, plants set little grain (Wiese, 1987) and stunted and chlorotic plants may appear in patches (Benedict and Mountain, 1956). *P. thornei* is associated with decreased wheat yields on the Darling Downs of Queensland (Colbran and McCulloch, 1965; Thompson *et al.*, 1981), and on the heavy black and grey clay soils of northern New South Wales (Doyle *et al.*, 1986, 1987). *P. thornei* is a major contributor to reduced wheat yield in Mexico (Van Gundy *et al.*, 1974), and in Canada, *P. neglectus* and *R. solani* are consistently associated with yellowed and stunted patches of wheat (Benedict and Mountain, 1956). However, wheat varieties can differ considerably in their reaction to *Pratylenchus* spp. (Van Gundy *et al.*, 1974; O'Brien, 1983; Thompson and Clewett, 1989).

P. neglectus has been associated with patches of poor growth in South Australian

cereal crops, although it has not been determined as the sole cause of patches, and is likely to be dispersed throughout a crop. This nematode has been identified in conjunction with several species of fungi (Kimpinski, 1972; Kimpinski *et al.*, 1976; Patel, 1983) in South Australian cereal crops. Stynes (1975) found that *P. neglectus* was ubiquitous in wheat crops across the Yorke Peninsula of South Australia, and in Victoria *P. neglectus* was more numerous in patches of poor growth, along with several species of fungi (Price, 1970).

Association With Fungi

Numerous records exist of interactions between fungal pathogens and nematodes, notably *Pratylenchus* spp. *Verticillium albo-atrum* and *P. penetrans* act synergistically in the wilt diseases of eggplant, tomato and pepper (McKeen and Mountain, 1960; Powell, 1963). *Cylindrocarpon radicicola* root rot of potato, carrot, red clover, tomato, spinach and violet is exacerbated in the presence of *P. pratensis* (Hastings and Bosher, 1938), and *P. penetrans* contributes to root rot of bulbs in Holland (Slootweg, 1956). *Pratylenchus* spp. are associated with roots of sugarcane infected by *Sclerospora* (Birchfield, 1953) or *Pythium* spp. (Croft *et al.*, 1985), and with strawberry root rots (Hildebrand and West, 1941; Klinkenberg, 1955; Dickerson and Slack, 1958).

A number of mechanisms whereby fungi and nematodes act in conjunction to cause root disease have been postulated. Root wounding incited by nematodes can expose root tissues to fungal invasion (Miller, 1956; Armstrong and Armstrong, 1958; Newhall, 1958), and Slootweg (1956) suggested that *P. penetrans* contributes to increased incidence of bulb rot by providing wounds through which fungi may enter. Although most fungal pathogens are capable of invading unwounded root tissues, it is possible that mechanical wounding is an important factor assisting fungal infection of roots (Powell, 1963). Fungi may then become more damaging in conjunction with nematodes (Pitcher, 1965), and the damage primarily caused by nematodes is compounded by the effects of these secondary parasites (Townshend and Anderson, 1976).

Nematodes may elicit a host response that lowers natural resistance to fungal invasion (Jenkins and Coursen, 1957). Physiological changes brought about by

nematode infection are probably more important in fungus-nematode interactions than is root wounding alone (Powell, 1963). The fungus may render roots more susceptible to nematode invasion (McKeen and Mountain, 1960), but the reverse is more likely to be the case with *Pratylenchus* spp. which do not tend to feed on already rotted tissues.

There are numerous examples of apparently synergistic relationships, or at least close associations, between *P. thornei* or *P. neglectus* and cereal root rot fungi (Benedict and Mountain, 1956; de Beer, 1965; Price, 1970; Kimpinski, 1972; Stynes, 1975; Patel, 1983; Stynes and Veitch, 1983), and many of these are South Australian based studies.

2.4 *BIPOLARIS SOROKINIANA*, *FUSARIUM* SPP. AND ASSOCIATED FUNGI

The term "common root rot" is usually applied to disease resulting from *Bipolaris sorokiniana* infection of the subcrown internode of wheat and barley, but the aetiology of this disease is extremely complex, and a number of fungi can be involved. This is especially true in South Australia, where *Fusarium* spp. are always isolated more frequently from subcrown internodes than is *B. sorokiniana*. In this case, the descriptor "common root rot" should be avoided, and reserved for situations where *B. sorokiniana* is the major incitant of disease. Harris (1987) suggests that, in South Australia, the disease complex should be referred to as "dryland root rot".

B. sorokiniana (Sacc.) Shoem. 1959 (syn. *Helminthosporium sativum* Pamm. *et al.* 1910, syn. *Drechslera sorokiniana* (Sacc.) Subram. and Jain 1966; teleomorph: *Cochliobolus sativus* (Ito and Kurib.) Drechsler ex Dastur 1942) and various *Fusarium* spp. Link ex Fr. 1821 jointly infect cereals, but the importance and frequency of these fungi varies regionally (Mathre, 1982; Hill *et al.*, 1983) and seasonally. These fungi are ubiquitous, non-discriminating pathogens (Wiese, 1987) with a wide host range (Gordon and Sprague, 1941; Andrews, 1943, 1953; Sprague, 1950). Sprague (1950) listed 102 gramineous species as hosts of *B. sorokiniana*, and Alcorn (1983) listed 41 host species. It is difficult to breed cereal varieties resistant to a disease caused by several species of fungi that have a wide host range, although resistance to individual components of the

disease complex is possible. Commercial varieties with resistance to *B. sorokiniana* or *F. graminearum* are available in South Australia, Queensland and New South Wales.

Of the cereals, wheat and barley are the most important hosts of *B. sorokiniana*, while oats are not susceptible. Many other grasses are reported to be just as vulnerable to *B. sorokiniana* as are wheat and barley (Diehl, 1983), and the fungus has been recorded on many grass species that commonly occur as weeds in cereal crops (Christensen, 1922; Hynes, 1923, 1932, 1935b; Carne, 1927; Sprague, 1946a; Andersen, 1955). In North America, gramineous weeds carry-over *B. sorokiniana* which then infects cereal crops (Padwick and Henry, 1933; Padwick, 1935), and weeds are also a major source of inoculum to cereals in Australia (Harris, 1987). *B. sorokiniana* can infect dicotyledonous species (Renfro, 1963; Graham *et al.*, 1964; Gourley, 1968), but is considered non-pathogenic to these plants.

Fusarium spp. occur ubiquitously in soil, on plant debris and in diseased tissues, and are among the most common fungi encountered by pathologists (Booth, 1971). By virtue of their worldwide distribution and the existence of many species, the fusaria cover an enormous host range. Booth (1971) recognises more than 50 *Fusarium* spp. However, there are over 1000 published names due to nomenclatural confusion, difficulties in identifying the different spore types of each species on various media, and the occurrence of *formae speciales* within individual *Fusarium* spp.

Aetiology

Reports of disease caused by *B. sorokiniana* and *Fusarium* spp. exist from all major cereal-growing areas of the world, although the relative importance of these fungi and the constitution of the *Fusarium* flora differs from place to place. *B. sorokiniana* is more commonly associated with the disease complex than are *Fusarium* spp. in the Canadian Prairie Provinces, the Soviet Union (Mathre, 1982) and North Dakota (Statler and Darlington, 1972). Skou (1967) identified *B. sorokiniana* as the primary cause of a severe disease of barley in Denmark. In contrast, *F. culmorum* and *F. graminearum* are more common than *B. sorokiniana* in the Mediterranean area of the Middle East, and the Pacific Northwest of the United States. *F. culmorum* and *F. graminearum* are also

involved in the disease in East Africa (Saari and Wilcoxson, 1974), California (Oswald, 1950; Scardaci and Webster, 1982) and Nebraska (Fenster *et al.*, 1972).

In California, Mackie (1931) identified "pink roots" caused by *F. culmorum*, but Oswald (1950) later reported that the disease was primarily due to infection with *B. sorokiniana* and *F. graminearum*. Scardaci and Webster (1982) frequently isolated *F. culmorum*, *F. graminearum*, *B. sorokiniana* and *Microdochium bolleyi* from the crowns and subcrown internodes of diseased wheat and barley plants in California, although the frequency of isolation of these components of the disease varied with the region in which the crop was grown. Oswald (1950) considered *F. culmorum* to be unimportant in most areas, but Scardaci and Webster (1982) indicated that this pathogen is an important component of the disease complex, widespread in California.

B. sorokiniana is of relatively minor importance in Britain (Salt, 1977). In 1929, Russell (1931) isolated *B. sorokiniana* from plants with rotted stem bases, but *F. culmorum* was the prevalent pathogen. Although *B. sorokiniana* infected only 2% of plants, Russell (1931) recognised the potential of *B. sorokiniana* to become damaging to cereals in Britain under conditions favouring disease development, and in combination with *Fusarium* spp. Plots inoculated with *B. sorokiniana* plus *F. culmorum* suffered more severe disease than those inoculated with *F. culmorum* alone.

Several species of fungi are involved in a root disease complex in South Africa. On the Western Cape, Gorter (1943) reported that root rot was caused by *F. graminearum*. In the Transvaal, Putterill (1924, 1940) attributed disease to *B. sorokiniana*. Jooste (1965) reported *B. sorokiniana*, *Rhizoctonia* and *F. culmorum* as the causal organisms of crown rot of wheat in the Highveld region. The species most frequently isolated by Maas and Kotze (1981) were *B. sorokiniana*, *Gaeumannomyces graminis*, *Periconia macrospinosa*, *M. bolleyi* and *Pythium oligandrum*. *B. sorokiniana* was detected in all areas sampled, although its incidence was low on the Western Cape, where *F. culmorum* and *F. graminearum* dominated.

B. sorokiniana is the major incitant of root rot on the Canadian Prairies (Sallans and Tinline, 1965; Harding, 1973; Verma *et al.*, 1974), where the disease can truly be regarded as "common root rot". Five species of fungi capable of causing crown and root

rot were identified in Manitoba by Sturz and Bernier (1987a): *B. sorokiniana*, *G. graminis*, *F. culmorum*, *F. avenaceum* and *F. nivale*. *M. bolleyi* and *F. equiseti* were also isolated frequently, but were regarded as non-pathogenic. In Saskatchewan, Simmonds and Ledingham (1937) found that *B. sorokiniana* constituted 10% of fungal isolates from wheat, and *Fusarium* spp. 40%. Harding (1973), however, isolated *B. sorokiniana* from about 80% of wheat subcrown internodes, and *Fusarium* spp. were isolated infrequently. In Alberta, disease is caused by a combination of *B. sorokiniana* and *Fusarium* spp. (Harper and Piening, 1974).

B. sorokiniana and *F. acuminatum* are the primary components of the root disease complex in Colorado and Wyoming (Hill *et al.*, 1983). *B. sorokiniana* comprised 34% of fungal isolates from wheat and *F. acuminatum* 28%. In Wyoming, Fernandez *et al.* (1985) found that *F. acuminatum* significantly augmented the effects of *B. sorokiniana* on wheat, especially following exposure to freezing temperatures. *B. sorokiniana* is the predominant cause of "common root rot" of wheat in Brazil (Diehl, 1979), and comprised 60% of the fungi isolated. *Fusarium* spp. made up the majority of other species encountered: *F. oxysporum* 20%, *F. graminearum* 5%, *F. avenaceum* 3%, *F. acuminatum* 2% and *F. equiseti* 2%.

The situation in Nigeria is somewhat different from that in other regions (Giha, 1976, 1978), as *Drechslera rostrata* and *F. equiseti* were the principal fungi infecting roots and stem bases. Other species were also isolated: *B. sorokiniana*, *Drechslera* spp., *F. acuminatum*, *F. semitectum* and *F. fusariodes*. Apart from *B. sorokiniana* and *F. acuminatum*, these fungi are not usually associated with cereal root disease. Although *D. rostrata* was the species most frequently isolated, it was less destructive than *B. sorokiniana*.

Infection of wheat by *B. sorokiniana* has been reported from every mainland state of Australia (Wildermuth, 1986). Moore and Herridge (1983) reported severe disease due to *B. sorokiniana* in central New South Wales where successive wheat crops were sown. Disease caused in part by *B. sorokiniana* is widespread in South Australia (Mayfield, 1981; Harris and Moen, 1981; Tinline, 1984). *B. sorokiniana* is responsible for minor yield reductions in Victoria (Price, 1970), and Chambers (1962) reported *B. sorokiniana*

in wheat suffering root rot in Western Australia.

B. sorokiniana is the major incitant of "common root rot" in Queensland (Purss, 1970), and is the most widely distributed soil-borne disease of wheat in that State (Wildermuth and McNamara, 1986). Simmonds (1966) first recorded *B. sorokiniana* in Queensland in 1964, and in the same year Ledingham (1966) found that 22% of plants in that State were infected by *B. sorokiniana*. *B. sorokiniana* was detected in all areas of Queensland in a survey conducted between 1978 and 1980 (Wildermuth, 1986).

Moen and Harris (1980) have isolated a wide range of fungi from wheat roots in South Australia. In 1978, they found *B. sorokiniana* and *F. culmorum* to be the most common species. *F. culmorum* and *F. solani* were prevalent in 1979 but, in 1985, *F. equiseti*, *F. acuminatum*, *F. oxysporum* and *B. sorokiniana* were isolated most frequently from wheat and barley crops (Fedel-Moen and Harris, 1987). *Fusarium* spp. infected the subcrown internode, crown roots and culm bases with equal frequencies on wheat and barley, whereas *B. sorokiniana* colonised culm bases and subcrown internodes and was isolated more often from barley than from wheat.

Early studies in New South Wales emphasised that *F. culmorum* accompanied *B. sorokiniana* in cereal root disease (Hynes, 1935b, 1937b, 1938), and *F. culmorum* is widely accepted as a frequent component of disease elsewhere (Butler, 1962; Harding, 1973; Tinline, 1976; Scardaci and Webster, 1982). Hynes (1937b) recognised that root rot was more pronounced when plants were infected by *B. sorokiniana* in conjunction with *Fusarium* spp. *F. culmorum* is, however, infrequent in Queensland (Purss, 1970) and Victoria (Chambers, 1972), and in South Australia occurs at high frequencies in only some years (Moen and Harris, 1980; Fedel-Moen and Harris, 1987). In South Australia, the role of *F. culmorum* is usually replaced by a mixed infection of *F. equiseti*, *F. acuminatum* and *F. oxysporum* (Fedel-Moen and Harris, 1987).

There are numerous reports identifying fungi besides *B. sorokiniana* and *Fusarium* spp. that are associated with cereal root rot. These fungi are largely ignored, but some workers recognise that such species may be important components of the disease complex. *B. sorokiniana* can predispose plants to infection by fungi that are normally considered weak pathogens (Ludwig *et al.*, 1956). In South Australia, Harris

(1986, 1987) identified a suite of fungi associated with rotted cereal roots: *M. bolleyi*, *Curvularia* spp., *Phoma* sp., *Embellisia* sp., *Athelia* sp., *Cylindrocarpon* sp., *Ulocladium atrum* and *Periconia macrospinosa*.

Curvularia ramosa was first recognised as a cereal root pathogen in Australia in the 1920's, under the name "*Helminthosporium M*" (Henry, 1924; Hynes, 1935a; Dickson, 1947). It was noticed in South Australia at about the same time (Samuel, 1924), but not established as a cereal root pathogen until the 1930's (Hynes, 1935b, 1936). Hynes (1935a, 1936) demonstrated that *C. ramosa* was more virulent than *B. sorokiniana*, although it was less frequently associated with cereal root disease. Sprague (1950) regarded *C. ramosa* to be similar, if not identical to, *C. geniculata* (Tracy and Earle) Boedijn 1933 (syn. *Cochliobolus geniculatus* R. Nelson 1964) which is sometimes associated with decay of cereal and grass roots in North America. *Curvularia* spp. are also associated with root rot in New Mexico (Hsi, 1956), Colorada and Wyoming (Hill *et al.*, 1983).

Curvularia spicifera (Bain.) Boedijn 1933 (syn. *Bipolaris spicifera* (Bain.) Subram. 1971) was first described by McKinney (1925) as *Helminthosporium tetramera*, and isolated from wheat in Australia in 1923 (Hynes, 1936). It has also been associated with rotted stem bases of cereals in India (Chattopadhyay, 1953), South Africa (Putterill, 1954), North America, Europe and Asia (Zillinsky, 1983). Hynes' (1935a, 1935b) isolates were only weakly pathogenic, and *C. spicifera* is more common as a saprophytic coloniser of seed and straw of cereals and grasses than as a true parasite (Crosier and Weimer, 1940; Sprague, 1950).

Periconia macrospinosa Lefebvre and A. G. Johnson 1949 is implicated in "crater disease" of wheat in South Africa (Scott *et al.*, 1979), but the importance of *P. macrospinosa* in the root disease complex in other areas is uncertain. Maas and Kotze (1981) isolated *P. macrospinosa* from wheat, in conjunction with other root rot fungi, in South Africa, and Hoes (1962, 1964) isolated this species from wheat in Washington. *P. circinata* has also been isolated from wheat roots in South Africa (Maas and Kotze, 1981) and Australia (Sims *et al.*, 1961).

Symptoms

The symptoms of disease caused by *B. sorokiniana* and *Fusarium* spp. can be rather non-specific and may be disguised by those of a more serious pathogen (Skou, 1967). Root damage is concentrated in the cortex and the fungi involved only rarely invade the stele (Butler, 1962). Roots show a general discolouration and rotting, which can spread to the crown and stem bases. Basal stem tissue invaded by *B. sorokiniana* may become coated with a dark, loose mass of spores and mycelium (Butler, 1962). Infected lower nodes appear brown to black in colour, resulting in restricted nutrient transport (Zillinsky, 1983). By maturity, the subcrown internode displays circular to striate, dark brown lesions extending into the crown. These subcrown internode lesions are characteristic of invasion by *B. sorokiniana.*

Seedling blight is the most dramatic result of early *B. sorokiniana* attack, causing seedling death before or soon after emergence (Wiese, 1987), but this is unusual under South Australian conditions. Surviving seedlings develop brown lesions on roots, coleoptiles and crowns. If enough lesions develop on the subcrown internode, these can elongate and coalesce to the extent that the entire subcrown internode becomes constricted and turns dark brown or nearly black. Roots may become dark brown and "stubby" (Skou, 1967) or stunted (Harris, 1986), and plants appear chlorotic (Russell, 1931), especially in the lower leaves. Chlorosis can result from impaired nitrogen metabolism (Simmonds, 1961), as *B. sorokiniana* intercepts nitrogen in the host plant, and may be responsible for reducing the protein content of grain (Simmonds, 1960). Plants can develop varying degrees of root rot at later growth stages (Hynes, 1937b). The primary roots are more prone to infection than the secondary roots (Hynes, 1935b), so plants may recover to some extent if secondary roots remain healthy. However, P. J. L. Whittle (personal communication) has observed lesions on the crown roots of wheat inoculated with *B. sorokiniana.* Initially, the fungus was isolated from superficial, brown streaks. Dark brown lesions then developed, penetrating the cortex, and finally rotting the root all the way through.

Plants infected with *F. graminearum* and *F. culmorum* display discoloured and rotted lower leaf sheaths, culms, crowns and subcrown internodes (Scardaci and

Webster, 1982). *B. sorokiniana* infection does not extend as far up the culm, and the fusaria often produce salmon coloured sporodochia further up the stem, especially under moist conditions. When fusaria are involved in disease, infected tissue may take on a pink to purplish-red cast, and infected culm tissue appears brown 3 - 10 cm above the soil line (Mathre, 1982). It is sometimes difficult to separate lesions on the subcrown internode caused by *B. sorokiniana* from those due to *F. graminearum* or *F. culmorum*, but these fungi usually occur together in mixed infections. *Fusarium* spp. invade secondary roots as these emerge from the crown and rotting proceeds throughout the growing season (Wiese, 1987). Infections that extend to the crown or stem base tissues are most damaging, and may be lethal under dry conditions. Culm bases and lower nodes become dry and darkly coloured, and diseased plants can become brittle and break off easily near soil level.

Above-ground symptoms are often indistinct in plants suffering only moderate infection by *B. sorokiniana* or *Fusarium* spp. (Mathre, 1982). *F. graminearum*, however, can cause "hay-die" symptoms, while *B. sorokiniana* is more insidious and results in reduced plant vigor, accelerated maturity and shrivelled grain. Infection may occur at any stage of development, resulting in pre- or post-emergent seedling blight and root, crown or stem base rot of older plants (Butler, 1962). Symptoms are usually more pronounced after heading, and root and crown rot infections that occur during flowering usually kill plants before seed formation (Zillinsky, 1983).

F. graminearum is often detected in general isolations from cereal roots, but is more important as the causal agent of "crown rot". Burgess *et al.* (1981) have described the process of infection by *F. graminearum* and the development of symptoms associated with the disease. Infection normally occurs through the subcrown internode, coleoptile or crown. Although *F. graminearum* is sometimes isolated from discrete root lesions, these are not regarded as primary infection sites. Plants are susceptible to infection at all growth stages, with the fungus progressively colonising the crown, tiller bases, lower leaf sheaths and proximal regions of seminal and crown roots. Extensive colonisation of the crown and stem bases is associated with distinctive honey-brown discolouration of tiller bases. Mycelium develops in the lumen of colonised stems, and externally under moist

conditions. Salmon coloured spore masses may also be observed on the stems. Plants ripen prematurely, and become apparent as bleached whiteheads.

"Crown rot" is not severe on well-drained, sandy soils (Burgess *et al.*, 1981), and primarily occurs on the heavy black and grey clay soils in the eastern States (Klein *et al.*, 1985). The disease is most severe when plants are subject to water stress (Cook, 1981a; Burgess, 1981), particularly when this occurs at anthesis or during grain filling (Klein *et al.*, 1985). Disease severity varies with seasonal conditions, time of sowing, soil type and nutrient status (Dodman *et al.*, 1985), all of which determine plant water use and thus the development of moisture stress (Cook, 1981b).

Yield Loss and Economic Significance

There are inherent difficulties associated with the accurate assessment of damage attributable to cereal root rots (Butler, 1962) and, usually, yield losses can only be estimated. However, disease rating, based on the severity of subcrown internode lesioning caused by *B. sorokiniana*, has been related to the magnitude of yield loss in wheat (Tinline *et al.*, 1975; Tinline and Ledingham, 1979). Pua *et al.* (1985) found significant correlations between the intensity of *B. sorokiniana* infection and yield loss in barley. Ledingham *et al.* (1973) recorded yield losses of wheat in three disease categories. Plants with slight subcrown internode lesioning had yields reduced by 6.0%, moderate lesioning reduced yield by 12.5% and severe lesioning led to a 28.2% reduction in yield.

B. sorokiniana deleteriously affects the yield components of cereal crops, reducing stand and tiller numbers, as well as plant height, head size and kernel weight and number (Ledingham *et al.*, 1973; Piening *et al.*, 1976; Pua *et al.*, 1985; Wiese, 1987). Ledingham *et al.* (1973) measured a 15.2% reduction in number of heads per plant, and a 6.7% reduction in 1000-kernel weight of severely diseased plants.

Yield losses in Canada due to "common root rot" are well documented, and an annual loss of 5.7% is estimated for the Canadian Prairies (Ledingham *et al.*, 1973; Wiese, 1987). In Manitoba, Machacek (1943) recorded an overall average reduction in wheat yields of about 12%. A ten year survey in Saskatchewan revealed an average

annual loss equivalent to approximately one third of the yields actually harvested (Sallans, 1948). Sallans and Ledingham (1943) determined an average wheat loss of 29%. These figures are more easily calculated in Canada than elsewhere, because *B. sorokiniana* alone is usually the major cause of the disease.

B. sorokiniana also causes considerable losses to barley in the Canadian Prairie provinces (Piening, 1973), which are greater than those in wheat (Piening *et al.*, 1976). In Saskatchewan between 1969 and 1971 wheat yields were reduced by 5.7%, and 7.1% in 1970-1971 (Ledingham *et al.*, 1973). Barley losses for the same periods were 10.3% and 14.4% respectively (Piening *et al.*, 1976). In Alberta, wheat yield was reduced by 6% (Ledingham *et al.*, 1973) and that of barley by 10.5% (Piening *et al.*, 1976) between 1970 and 1971.

Yield reductions reported from the United States are generally lower than those from Canada, and wheat losses of only 3 - 4% are attributed to *B. sorokiniana* and associated fungi (Wiese, 1987). Sprague (1948a) estimated a loss of 4% in Washington, although higher losses (16 - 21%) occur in Pennsylvania where crops are subjected to freezing temperatures during winter (Frank, 1985).

Root rot was devastating in New South Wales in the 1920's and early 1930's when wheat monoculture was practised, and individual crop losses of 25% and greater were not uncommon (Hynes, 1932). In Queensland, Wildermuth and McNamara (1986) recorded yield losses due to *B. sorokiniana* infection of wheat ranging from 2.4% to 22%, depending on varietal susceptibility. In 1983, Wildermuth (1985) recorded losses of 34% in one susceptible wheat variety, and even Kite, the most resistant commercial variety, suffered a loss of 19%. Losses in South Australia have been estimated at 5 - 10% for wheat and barley, but this is influenced by seasonal conditions (P. J. L. Whittle, personal communication). In 1988, P. J. L. Whittle recorded a 16% yield loss in Machete wheat (susceptible to *B. sorokiniana*) and no loss in moderately resistant varieties.

Yield losses due to "crown rot" (*F. graminearum*) in Queensland have been recorded at up to 26% in individual crops, but an overall figure of approximately 5% has been estimated (Burgess *et al.*, 1981). This disease is more important in northern New South Wales and southern Queensland (Burgess *et al.*, 1981; Dodman *et al.*, 1985; Klein

et al., 1985) than it is in South Australia. A random survey by T. A. Klein (personal communication) showed that "crown rot" was of little significance in South Australia, but could severely affect individual crops. At the Charlick Experiment Farm (Strathalbyn), 84% of wheat plants in one field showed basal browning, which caused an estimated yield loss of 51%. In the Murray Mallee, approximately 30% of plants in one crop showed severe symptoms of "crown rot".

2.5 *MICRODOCHIUM BOLLEYI*

Microdochium bolleyi (Sprague) de Hoog and Hermanides-Nijhof 1977 (syn. *Gloeosporium bolleyi* Sprague 1948; syn. *Aureobasidium bolleyi* (Sprague) v. Arx 1957) is extremely common in agricultural soils (Domsch *et al.*, 1980), particularly in conjunction with *Bipolaris sorokiniana* and associated species that cause root rot, but is generally regarded as a "minor" pathogen. *M. bolleyi* has been isolated from cereals, grasses and other species worldwide, where it is frequently the most common fungus recorded. However, the role of this species in cereal root disease is not clearly understood, and there are conflicting reports regarding its pathogenic potential.

Occurrence

Sprague (1948b) isolated *M. bolleyi* from the roots and crowns of 121 gramineous species, and commented that the host range of this fungus seemed to be "virtually unlimited". *M. bolleyi* constitutes a large proportion of the fungi isolated, except in drier years (Sprague, 1948b), when *B. sorokiniana*, *F. oxysporum* and *R. solani* may be more common (Sprague, 1944).

Hoes (1964) found that this species was one of the first to colonise healthy plants. Kane *et al.* (1987) consider *M. bolleyi* to be a potentially important, but minor, component of a root disease complex, although it is frequently isolated from cereals suffering root rot in California (Scardaci and Webster, 1982), Washington (Hoes, 1962, 1966), Montana (Sharp, 1959), New York (Kane *et al.*, 1987) and Manitoba (Sturz and Bernier, 1985, 1987a).

In West Germany, *M. bolleyi* is a frequent invader of the stem bases of cereals, especially wheat (Reinecke, 1978; Reinecke and Fokkema, 1981). Domsch and Gams (1970) and de Hoog and Hermanides-Nijhof (1977) frequently detected *M. bolleyi* on wheat roots and subcrown internodes. In conjunction with *Fusarium* spp. and other fungi, *M. bolleyi* was also isolated from diseased maize roots in West Germany (Kruger, 1976).

M. bolleyi colonises wheat (Salt, 1977) and barley (Slope and Broom, 1974; Salt, 1977; Murray and Gadd, 1981) roots in Britain, where it has been found to infect more than 50% of root segments tested (Salt, 1977). Murray and Gadd (1981) regularly recovered *M. bolleyi* in conjunction with *R. solani* from stunted patches of barley in Scotland, although *M. bolleyi* was not responsible for stunting, nor did it increase the severity of disease caused by *R. solani*. *M. bolleyi* was the most frequent species isolated from cereals by Waller (1968) in Britain, where it was more common on barley than on wheat (Waller, 1968, 1979).

With the exception of the Springbok Flats (where soil temperatures are as high as 30°C during the growing season), all major wheat producing areas of South Africa are infested with *M. bolleyi* (Maas and Kotze, 1981). In Poland, *M. bolleyi* is one of the fungi most frequently associated with winter rye (Blotnicka and Kocerba, 1977), and has also been found in the soil of Polish potato fields (Choroszewski, 1985). Tichelaar (1978) found that stunted wheat plants in the Netherlands were infected by *M. bolleyi*, and in Sweden *M. bolleyi* was the most frequent species isolated from barley roots (Jonsson, 1987).

Little information regarding *M. bolleyi* is available from Australia. However, in South Australia, Harris (1986) found that *M. bolleyi* was associated with rotted cereal roots in the field, and with roots that had been originally inoculated with *R. solani* (Harris and Moen, 1985a, 1985b; Moen and Harris, 1985).

Symptoms

M. bolleyi can cause necrosis and discolouration of the subcrown internode, which may be confused with mild symptoms of *F. graminearum*, *F. culmorum* or *B.*

sorokiniana infection (Scardaci and Webster, 1982). Broom (1972) isolated *M. bolleyi* from barley roots with pale brown, dark brown or black lesions, while Waller (1979) found that soft rot lesions, usually attributed to *Pythium* spp., yielded high levels of *M. bolleyi*. Thick-walled, black chlamydospores are often obvious in root cortical and epidermal cells (Murray and Gadd, 1981; Murray, 1981), but not in the stele or coleoptile (Murray and Gadd, 1981). These studies described orange-brown lesions on the coleoptile, which contained hyphae of the fungus.

Fitt and Hornby (1978) detected stunted and brown root axes only one week after plants had been inoculated with *M. bolleyi*, and most seminal roots suffered vascular disruption. These plants also had fewer, shorter leaves than uninoculated plants. *M. bolleyi* can decrease plant dry weight (Fitt and Hornby, 1976) by as much as 26% (Domsch and Gams, 1968). However, infected plants prematurely produced new crown roots which were never more than superficially infected (Fitt and Hornby, 1978). Plants thus recovered and *M. bolleyi* had no significant effect on plant growth by five weeks of age. *M. bolleyi* has, however, been reported to cause pre-emergence seedling death, and infected wheat may display stunted culms, "stubbed off" roots and a brown, decayed seed (Sprague, 1948b).

M. bolleyi infection can be associated with various symptoms, but the fungus is often as frequent on healthy plants as it is on diseased plants (Hoes, 1962, 1966; Waller, 1968, 1979; Murray, 1981; Murray and Gadd, 1981), although diseased barley plants tend to carry a higher population of *M. bolleyi* than do healthy plants (Waller, 1968, 1979). This suggests that *M. bolleyi* may be acting parasitically rather than strictly as a pathogen, and casts some doubt on reports that the fungus damages roots. However, isolates may differ in pathogenicity, with biotic and abiotic factors influencing the effect this fungus has on plants.

The presence of wounds is not necessary for the entry of *M. bolleyi*, and hyphae enter tissues by direct penetration and through stomatal openings (Murray and Gadd, 1981). Coleoptile tissues begin to decay only after entry of hyphae. Subsequent browning and cell disintegration are probably due to enzymatic activity of the fungus, or to substances produced by the host in response to infection, or both (Murray and Gadd,

1981). *M. bolleyi* shows high enzymatic activity in the breakdown of pectin, xylan and carboxymethyl-cellulose (Domsch and Gams, 1969).

Pathogenicity

Despite reports that *M. bolleyi* can be damaging to plant roots, it is generally considered non-pathogenic or only weakly so (Kruger, 1976), and fulfils the criteria for a "minor" pathogen as described by Salt (1979). "Minor" pathogens are restricted to juvenile tissues such as root hairs, root tips and cortical cells. These fungi are widely distributed in cultivated soils and have a wide host range. Distinctive symptoms are often lacking, and such species usually occur in mixed infections with other fungi.

Some of Sprague's (1946b, 1946c, 1950) isolates were pathogenic, but their behaviour was not consistent, and further tests indicated that none were pathogenic to Gramineae (Sprague, 1946b). Reinecke (1978) observed no symptoms on the stem base of inoculated wheat, although *M. bolleyi* was readily re-isolated from almost every plant. In the field, there were no symptoms attributable to *M. bolleyi,* and it had no significant influence on yield. Reinecke (1978) concluded that *M. bolleyi* was not pathogenic to cereals, although it was a common invader of the stem base.

Murray and Gadd (1981) observed limited damage to coleoptiles and roots, despite heavy colonisation by *M. bolleyi*. Wheat yield is not affected by *M. bolleyi*, although subcrown internodes develop small lesions (Kane *et al.*, 1987). Roots of wheat and cocksfoot (*Dactylis glomerata*) emerge and grow normally in the presence of *M. bolleyi* (Kirk and Deacon, 1987b), with the depth of fungal invasion paralleling the amount of natural cortical senescence. Living cells are penetrated poorly, and resting spores (chlamydospores) are produced in the vicinity of living cells. Kirk and Deacon (1987b) concluded that *M. bolleyi* is a weak parasite, invading senescent cortical cells or very young tissues, where there is little resistance to infection.

M. bolleyi isolates are less virulent to wheat (Sturz and Bernier, 1987a, 1987b) and barley (Scardaci and Webster, 1982) than those of other root rotting fungi (including *G. graminis*), although it is moderately pathogenic to barley (Scardaci and Webster, 1982), causing slight lesioning (Slope and Broom, 1974). "Minor" pathogens like *M.*

bolleyi can, however, become quite damaging under conditions of host plant stress (Harris, 1987). *M. bolleyi* is pathogenic to wheat seedlings in Manitoba stressed by freezing, where soil temperatures may be as low as - 20°C (Sturz and Bernier, 1987b). In the Netherlands, *M. bolleyi* was associated with yield reductions in wheat crops on light, sandy soils (Tichelaar, 1978). Black and Brown (1986) found that *M. bolleyi* was pathogenic to flax, wheat, barley, cabbage, oil seed rape and pea only after prolonged periods of wet weather.

The presence of other disease-causing organisms may predispose plants to infection by *M. bolleyi*. Toxins produced by *B. sorokiniana* can promote invasion by microorganisms that are normally considered to be non-pathogenic (Ludwig *et al.*, 1956), and infection by *R. solani* is followed by a succession of "minor" pathogens, including *M. bolleyi* (Harris and Moen, 1985a, 1985b).

2.6 *PYTHIUM* SPP.

There are numerous species of *Pythium* Pringsheim 1858 distributed worldwide, and most are capable of parasitising seeds and roots of a wide range of plants (Robertson, 1980). Dick and Ali-Shtayeh (1986) isolated 45 taxa in their study of *Pythium* distribution and frequency at four sites in Britain. In wheat-growing areas of Washington and Idaho, Chamswarng and Cook (1985) detected ten *Pythium* spp., and they suggested that these fungi were indigenous to the native grasses which dominated prior to wheat monoculture. At least nineteen species exhibit some degree of pathogenicity to wheat (Wiese, 1987), but many saprophytic species are also isolated from the roots of cereals and other grasses (Sprague, 1950). The existence of a plethora of species has compounded the problem of controlling disease caused by *Pythium* spp.

Many species of *Pythium* occur on wheat and barley in Australia (Ledingham and Vanterpool, 1967; Bratoloveanu and Wallace, 1985). Bratoloveanu (1985) isolated eleven species from barley-growing areas in South Australia, with *P. irregulare*, one of the most widespread and pathogenic of the pythia (Sprague, 1950), the dominant species. These fungi are considered to cause yield losses in South Australian barley crops (Bratoloveanu

and Wallace, 1985), although some species are only mildy pathogenic, if at all (Bratoloveanu, 1985). According to Mayfield (1981) and Murray and Brown (1987), *Pythium* spp. cause only slight damage and yield loss to wheat in South Australia.

Pythium spp. are most destructive in wet soil (Wiese, 1987) as they require high soil water content to infect roots (Domsch *et al.*, 1980). This was demonstrated by Bratoloveanu (1985), who found a strong positive correlation between soil moisture and the incidence of disease on barley seedlings. Populations of *Pythium* spp. were also higher during cooler months than in summer, and at the cooler and wetter of the sites surveyed (Bratoloveanu, 1985).

Symptoms

Infection by *Pythium* spp. leads to pre-emergence seed decay or damping-off of cereals and other grasses, causing seedlings to collapse from soft stem rot and root rot soon after emergence (Sprague, 1950). Browning root rot induced by *Pythium* spp. is characterised by a reduced root mass with brown lateral roots and cortical tissues (Wiese, 1987). Plants are stunted and chlorotic with few tillers, heading and maturity are delayed and heads are smaller and poorly filled. Severe infection results in a general root necrosis. These symptoms are caused by one or more species acting singly or in combination, and are more evenly distributed throughout the crop than are those of diseases such as *Rhizoctonia* "bare patch" or "take-all" (Wiese, 1987). However, *Pythium* root rot will presumably be more prevalent in wetter areas of the paddock. It is difficult to identify *Pythium* infection from root symptoms alone. Isolations must therefore be made on selective media, although this does not define the pathogenicity of the many species that may be detected.

Association With Other Pathogens

Pythium irregulare and *Pratylenchus thornei* occur together on barley at the Waite Institute, but appear to act independently in terms of host response to infection (Bratoloveanu, 1985). *P. irregulare* had a more damaging effect on plants than did *P. thornei*. Singh (1984) claimed that a low level of *P. irregulare* significantly increased the

number of *P. thornei* in wheat roots, but this is unlikely to occur under field conditions. *Pratylenchus* spp. vacate rotted roots (Dropkin, 1989), where fungal infection interferes with nematode activity (Patel, 1983; Walia and Gupta, 1986). Jenkins (1948) reported that colonisation by root rotting fungi was secondary to invasion by nematodes on small grains in Virginia, USA.

Pythium spp. are often isolated from the rotted roots of cereals, in conjunction with other species of fungi. In South Africa, *P. oligandrum* was one of a mixture of species isolated from wheat roots (Maas and Kotze, 1981). *P. ultimum* in Victoria (Price, 1970) and *P. irregulare* in Western Australia (Roberts and Sivasithamparam, 1986, 1987) have been isolated, among other pathogens, from cereal "bare patches". Pittaway and Rathjen (1984) found that *Pythium* spp. were among the pathogens most frequently isolated from wheat roots in South Australia, where they occurred in conjunction with many other species of root rotting fungi.

Pythium spp. are isolated rarely in general studies of plants and soil, primarily due to the isolation techniques employed (Domsch *et al.*, 1980). These fungi are sensitive to the surface-sterilants used to treat plant material prior to plating out on agar, and to the antibiotics in isolation media (Sprague, 1950; Domsch *et al.*, 1980). Their occurrence is thus underestimated or overlooked.

2.7 *RHIZOCTONIA SOLANI*

Rhizoctonia solani Kühn 1858 (teleomorph: *Thanatephorous cucumeris* (Frank) Donk 1956) is associated with the development of "bare patch" in South Australian cereal crops. The fungus has a wide host range and occurs virtually worldwide, but *R. solani* is recognised as causing a well-defined root rot of wheat only in Australia (Samuel, 1923; Samuel and Garrett, 1932; Hynes 1933). However, a similar disease is described as "crater disease" in South Africa (Scott *et al.*, 1979; Deacon and Scott, 1985). Since Samuel (1928; Samuel and Garrett, 1932) recognised this fungus as a cause of disease in South Australian cereal crops, much research has gone into its local biology and control. No genetic resistance to *R. solani* has been detected, but cultivation seems to reduce the

incidence of disease (Neate, 1984; Rovira, 1987).

Symptoms

Patches generally appear in the same position each year, and are more or less circular with sharply defined edges (Kerr, 1955). These patches are well-defined (Hynes, 1937a), so there is no gradation from healthy to diseased plants. Plants within patches, when these plants survive, are extremely stunted and chlorotic and their maturity is delayed (Samuel and Garrett, 1932; Hynes, 1937a; Roberts and Sivasithamparam, 1986; Wiese, 1987). Seminal roots are poorly developed and the coronal roots stunted near the crown: these diseased roots are readily decorticated (Hynes, 1937a). Root tips often appear reddish-brown and taper to a diagnostic "spear" point (Wiese, 1987). If plants are not killed as a result of attack at the seedling stage, they can ultimately produce functional crown roots and, if these roots do not become severely infected, plants are able to recover to some extent, but reach maturity later than uninfected plants (Samuel and Garrett, 1932; Hynes, 1937a).

Aetiology

Both pathogenic and non-pathogenic isolates of *R. solani* occur in cereal crops. Three pathogenic strains have been reported from South Australian cereal fields (Neate, 1985), where they occur more frequently within patches than outside patches (Kerr, 1955; de Beer, 1965). Kerr (1955) and de Beer (1965) concluded that "bare patch" results from a localised increase in hyphal concentrations of pathogenic strains, whereas *R. solani* populations outside patches are too low to cause appreciable disease. *R. solani* persists saprophytically in the soil (Butler, 1962), and even pathogenic strains are isolated more frequently from the soil than from root tissues (Kerr, 1955). Species of *Rhizoctonia* other than *R. solani* are also common in some South Australian wheat fields (Neate, 1985).

Plants infected by *R. solani* may be more readily invaded by other fungi (Hynes, 1937a). Harris and Moen (1985a, 1985b) in South Australia found organisms other than *R. solani* associated with diseased roots that displayed characteristic symptoms of *R.*

solani infection. A range of secondary parasites supplanted the initial *R. solani* infection (Harris and Moen, 1985a, 1985b; Moen and Harris, 1985), and this was true of five *R. solani* isolates tested. *Fusarium* spp. and *B. sorokiniana* infected plants by the time of tillering, and this later gave way to a community dominated by *M. bolleyi*. Several other species of fungi were also involved: *Trichoderma viride*, *Alternaria alternata, Periconia macrospinosa*, *Penicillium* spp. and *Aspergillus* spp. Many of these fungi are classified as "minor pathogens" (Colhoun, 1979; Salt, 1979), but should not be overlooked as sources of damage to roots (Moen and Harris, 1985). Moen and Harris (1985) concluded that *R. solani* is a primary pathogen of juvenile tissues, unable to maintain aggressive pathogenicity as plants mature. *R. solani* may be a poor competitor with other inhabitants of the rhizoplane, or require continuous re-infection from propagules in the soil (Harris and Moen, 1985b). However, these inoculation experiments were conducted in pots of field soil, in which growing conditions can interfere with the root rotting potential of *R. solani*. Cores of undisturbed field soil from "bare patches" are usually needed to reproduce the symptoms of *R. solani* infection that are observed on field-grown plants (Dubé, 1971).

The severity of the initial *R. solani* infection seems to determine the intensity of subsequent secondary infections. According to Moen and Harris (1985), this diverse range of associated organisms causes more damage than *R. solani* alone, and may be the ultimate cause of crop loss. When "minor" pathogens were excluded, Moen and Harris (1985) found that plants recovered from *R. solani* infection and suffered negligible damage. *R. solani* may be, nevertheless, an important component of the disease complex, as it incites root damage at an early stage of growth, allowing invasion by a succession of secondary parasites. It is critical that *R. solani* damage in the field is not inferred from symptoms alone, because subsequent infection by secondary organisms is equally important (Harris and Moen, 1985a, 1985b). In the field, the majority of *R. solani* strains, especially those that are non-pathogenic, probably give way to such a succession of secondary organisms.

In Western Australia, Roberts and Sivasithamparam (1986, 1987) also recognised that *R. solani* was but one part of a disease complex, where mixtures of fungi caused

more severe disease. A combination of *R. solani*, *Rhizoctonia* sp., *F. graminearum*, *Pythium irregulare* and *B. sorokiniana* resulted in the highest root disease index (Roberts and Sivasithamparam, 1987). Similarly, in South Africa, Scott *et al.* (1979) isolated fungi other than *R. solani* from the roots of wheat plants in patches: *P. macrospinosa*, *Pythium oligandrum* and *R. cerealis*. They concluded that *R. solani* contributes to the severity of disease, but could offer no conclusive evidence that it was the primary cause of the observed patches. Patel (1983) investigated patches of poor growth in South Australian cereal crops, but concluded that the occurrence of these patches could not be solely attributed to the pathogens she detected, although fungi and nematodes did occur at higher levels on plants within the patches. Price (1970) conducted soil analyses and found no nutritional explanation for the occurrence of patches of stunted plants.

Nematodes Associated With Rhizoctonia solani

It seems virtually impossible to relate the occurrence of patches of poor growth to root infection by a particular organism. Several comprehensive surveys of the occurrence of organisms in patches of poor growth have been conducted in South Australia. Kimpinski (1972) found a higher incidence of *R. solani* and *Gaeumannomyces graminis* within patches than in areas of normal crop growth, and Patel (1983) isolated *Heterodera avenae*, *G. graminis* and *R. solani* from patches. In Victoria, Price (1970) detected *F. culmorum*, *Pythium* spp., *Curvularia inaequalis*, *B. sorokiniana*, *R. solani*, *G. graminis* and *F. graminearum* on the roots of stunted plants within patches. The above-mentioned surveys also revealed the presence of the root lesion nematode *P. neglectus* within patches, where it was more common than on plants outside patches of poor growth. On the Yorke Peninsula of South Australia, Stynes and Veitch (1983) showed that numbers of *P. neglectus* in wheat roots at three and eight weeks were correlated with levels of *R. solani* and *G. graminis*, respectively, at anthesis. However, these fungi occurred relatively infrequently, and their presence was inferred from the appearance of symptoms on the roots. Nevertheless, early invasion by *P. neglectus* may foster the establishment of *G. graminis* and *R. solani* within the roots.

Benedict and Mountain (1956), in Canada, found a consistent association between

R. solani and *P. neglectus* on the lesioned roots of wheat within patches of chlorotic and stunted plants. When both organisms were controlled by application of methyl bromide, wheat growth was twice that achieved when either pathogen alone was controlled, suggesting that the effects of both organisms are necessary for full disease expression (Benedict and Mountain, 1956). It was thus suggested that the nematode aided root colonisation by the fungus. In South Australia, de Beer (1965) also found that *P. neglectus* was associated with *R. solani* in patches.

A similar result was obtained by Meagher and Chambers (1971) in Victoria. They found that a combination of *R. solani* and cereal cyst nematode (*H. avenae*) had a significantly greater effect on wheat growth than did either pathogen alone. This is, however, doubtful under field conditions, as invasion of roots by the fungus interferes with the nematode's ability to feed within the roots. Patel (1983) showed that roots were unsuitable for *H. avenae* if they were already infected with *R. solani*.

De Beer (1965) found that *R. solani* patches occurred more frequently on sandy than on heavy soils, even in the same paddock. Similarly, nematodes are affected by soil type, which influences moisture content (Kable and Mai, 1968). This in turn determines host vigor (Grandison, 1972) and nematode activity (Kable and Mai, 1968; Grandison, 1972; Kimpinski, 1972). Most plant parasitic nematodes are more damaging in sand than clay soils (Wallace, 1963), and *Pratylenchus* spp. are more numerous in sandy soils where they cause more severe damage to the host (Endo, 1959; Sher and Bell, 1965; Kable and Mai, 1968).

2.8 *GAEUMANNOMYCES GRAMINIS*

Gaeumannomyces graminis (Sacc.) v. Arx and Olivier 1952 is one of the most thoroughly studied of the root rotting fungi (Sprague, 1950). Butler (1962) considered it to be the most important of the root diseases of wheat, and *G. graminis* was once ranked second only in importance to stem rust as a cause of yield loss in wheat on a worldwide basis (Garrett, 1942). The disease "take-all" was first recognised in South Australia as early as 1852 (Anon., 1868), and McAlpine (1904) showed that the symptoms of "take-

all" and whiteheads were caused by infection of roots and tiller bases by *G. graminis* (then called *Ophiobolus graminis*). The fungus is distributed worldwide in temperate cereal-growing areas, where it is harboured by a range of grass species (Garrett, 1942). Like most root diseases, damage from *G. graminis* infection tends to be greater on sandy soils, and this was demonstrated by Samuel (1923) in the Pinnaroo district of South Australia. However, *G. graminis* is often troublesome on the heavier soil at the Waite Institute. Despite the wealth of research into this disease, no resistant wheat cultivars are available. In fact, genes for resistance to *G. graminis* probably do not exist in wheat, due to inadequate selection pressure on plants to evolve such genes (Wallace, 1987).

In the past, under intensive cereal cropping systems, this disease did reduce yields of individual crops in Australia by 25 - 75% (Richardson, 1910; Hynes, 1935b). MacKinnon (1920), however, estimated an average annual wheat yield loss of 7% due to "take-all", and Geach (1932) estimated the annual loss, on an Australia-wide basis, at only 2.5%. Because of improved cultural practices (heavy grazing of pasture residues, use of legumes in rotations, control of gramineous weeds in crops and pastures), losses due to "take-all" are now probably even lower than those recorded in the 1920's and 1930's. Furthermore, the phenomenon of "take-all decline" has reduced the severity of disease since cereal cropping began in Australia. With intensive cereal culture, a soil microflora antagonistic to *G. graminis* develops. This has been reported from most cereal-growing countries, although "take-all decline" seems less stable in Australia than it is in Europe (Rovira and Wildermuth, 1981).

Symptoms

Stunted and unthrifty plants occur in irregular patches, but infected plants may be distributed throughout the crop (Butler, 1962). The expression of the disease at the seedling stage,"take-all", results from infection of the seminal roots at or just below the seed. Plants appear chlorotic and stunted, with reduced tillering (Wiese, 1987) and black, stelar lesions in the seminal roots. If enough water is available, infection spreads into the crown and up the culm base, resulting in the production of a characteristic dark, shiny mycelial plate surrounding the culm base beneath the lowest leaf sheath (Butler, 1962;

Wiese, 1987). This induces the "hay-die" phase of the disease. Under South Australian conditions, these plants usually become obvious between heading and grain maturity, when they die prematurely and appear as bleached whiteheads. Many plants within a crop, however, suffer only mild root infection.

2.9 CONCLUSION

Most of the organisms covered in this review have a relatively wide host range, and invariably occur in mixed infections on cereal roots. It is therefore difficult to relate the presence of these organisms to the level of damage incurred by field-grown plants, especially as they sometimes occur on apparently healthy plants as well as in diseased roots. Furthermore, pathogenicity tests under controlled conditions can provide inconsistent results, and are no indication of the potential of an organism to produce disease under field conditions.

Throughout South Australia, especially on the sandier soils, the root systems of wheat plants suffer extensive damage. In some instances, root damage is so severe that wheat plants lodge prior to harvest. This is causing widespread concern to farmers and district agricultural advisers (Ragless, 1990). Root damage is not clearly attributable to any of the individual pathogens discussed above, nor to those currently regarded as the "major" pathogens of wheat in southern Australia: cereal cyst nematode, *G. graminis* and *R. solani* (Rovira, 1987). The impression is clear, however, that wheat roots are infected by a vast mycoflora, and this mycoflora is accompanied by nematodes (*Pratylenchus* spp.). It is not known to what extent root damage in the field can be attributed to any of these organisms, alone or in combination.

CHAPTER 3

GENERAL METHODS

3.1 POT EXPERIMENTS

Soil

Soils used in inoculation experiments were collected from the cultivated layer of the A horizon of typical wheat-growing properties. Plants were thus grown under similar nutritional and physical conditions to field grown plants. A 1:4 (v/v) mixture of loam, collected near Windsor (70 km north of Adelaide), and coarse sand was steam-sterilised at 70°C for one hour, for use in inoculation experiments described in Chapter 7. This soil mixture has been successfully employed in inoculation experiments with cereal root pathogens by J. R. Harris and R. Moen (personal communication). A similar soil from the Murray Mallee region (65 km east of Adelaide; Figure 3.1), where field experiments were conducted, was treated in the same way, and used in the inoculation experiments described in Chapters 8 and 11.

Heat-formed toxins are often produced in soils treated at high temperatures, resulting in poor plant growth, stunted roots and the development of dark lesions on the roots (Dawson *et al.*, 1965; Rovira and Bowen, 1966). Treatment at 60 - 70°C does not deleteriously affect plant growth (Dawson *et al.*, 1965), and heating soil to 70°C eliminates most pathogens, as well as saprophytes that may be antagonistic towards pathogens (Bollen, 1985). Even the thick-walled oospores of *Pythium* spp. are killed at 70°C, whereas treatment at 50°C only reduces their germinability (Stasz and Martin, 1988). Insects, mites and most weeds are also eliminated by treatment at approximately 70°C (Blom *et al.*, 1988). Addition of sand to the soil mix reduces the risk of chemical toxicities (J. R. Harris, personal communication), such as those caused by manganese and nitrite ions (Dawson *et al.*, 1965).

Pots

Hygienic Lily® white plastic food containers with a capacity of approximately 650 g of soil were used for all inoculation experiments. Pots were 10.5 cm high and 9.0 cm in diameter. These pots did not have drainage holes.

Growing Conditions

After planting, soil was firmly tamped down, and all pots watered with equal volumes of distilled water. Pots were then placed in the glasshouse in a waterbath maintained at 19±2°C. Plants were watered with distilled water as required.

Inoculation

Microdochium bolleyi isolate #3071 and *Bipolaris sorokiniana* isolate #3061 were used in all inoculation experiments. *M. bolleyi* #3071 was originally isolated on December 2, 1986 from a lesioned wheat subcrown internode collected from B. Ramm's property, Mannum, Murray Mallee (Figure 3.1). *B. sorokiniana* #3061 was isolated on September 3, 1986 from a severely lesioned subcrown internode of Halberd wheat collected on B. Allen's property, Kimba, Eyre Peninsula.

Cultures used for inoculum were always those that had been recently re-isolated from inoculated wheat plants. Colonies were grown on plates of Difco® potato dextrose agar (PDA), incubated at 20 - 25°C for two to three days, then placed under a light bank at room temperature to induce sporulation. The light bank consisted of two white fluorescent tubes and a black light of 350 nm wavelength. Only those sporulating extensively and free of contaminants were used, and *M. bolleyi* colonies that had formed chlamydospores were avoided. Colonies were cut into 1.0 cm^2 segments, which were thoroughly mixed on a magnetic stirrer with sterile 0.1% NaCl solution in distilled water to disperse spores. Remaining agar squares were removed from the spore suspensions.

Numbers of *M. bolleyi* spores were not determined, but all suspensions were heavily loaded with the small spores, and were administered to pots in equal amounts. *B. sorokiniana* spore numbers were calculated by diluting small quantities of the resulting spore suspension and adding this to PDA plates, where it was spread evenly over the agar

surface. After incubation at 20 - 25°C for 24 hours, resulting colonies were counted, and spore concentrations calculated. *B. sorokiniana* spores were then used at a concentration of approximately 300 spores/gram of soil to inoculate pots.

Seed Sterilisation

Wheat seed was surface-sterilised prior to planting, using the method of Speakman and Kruger (1983). Seed was:

(1) soaked in 10 ppm Terramycin (oxytetracycline hydrochloride) solution for twenty hours,

(2) immersed for ten minutes in 0.1% $AgNO_3$ solution,

(3) rinsed in 0.5% NaCl solution to precipitate Ag^+ ions out of the solution, and

(4) washed in three changes of sterile distilled water (SDW).

All solutions contained 0.06% wetting agent Tween 20 (monolaurate), and were prepared in distilled water.

Germination of Seed

Seed was pre-germinated on PDA or tapwater agar (TWA) plates at 20 - 25°C for two to three days prior to planting. Any remaining contaminants on or in the seed could then be detected by their growth on the agar, and such contaminated seeds were discarded. Only healthy seedlings with three roots at least 1.0 cm long were chosen for planting. In all experiments, five pre-germinated seeds were planted in each pot.

Sampling

Soil was washed from roots under running tapwater. Plants were then placed, in distilled water, on a white tray to observe symptoms and remove root samples from which isolations were made. Root segments of 1.0 cm length and entire subcrown internodes were removed and stored in vials of SDW at 5°C until plating. Twelve root segments were removed randomly (ie. not specifically selected for the presence of visible symptoms) from each replicate, and six placed on each of two Petri dishes of isolation medium. All subcrown internodes from each sample were similarly placed on plates of

the isolation medium. Roots and shoots from each pot were dried separately in a forced-air dehydrator at 80°C for at least 48 hours prior to being weighed.

3.2 FIELD EXPERIMENTS

Sites

All field experiments were conducted in the Murray Mallee region of South Australia, approximately 65 km east of Adelaide. The following farmers' properties at these sites were used:

1987 - K. Maxwell, Sanderston and B. Ramm, Mannum

1988 - P. Royal, Sanderston and B. Ramm, Mannum

1989 - D. Abraham, Caloote and B. Ramm, Mannum.

Additional experiments sown in late August of the 1989 growing season were planted on the properties of B. Ramm, Mannum and D. Schirmer, Cambrai. Barley plants were sampled from M. Kluge's property, Purnong, in 1986. The location of all these sites is mapped in Figure 3.1. All sites are in the rain shadow of the Mount Lofty Ranges.

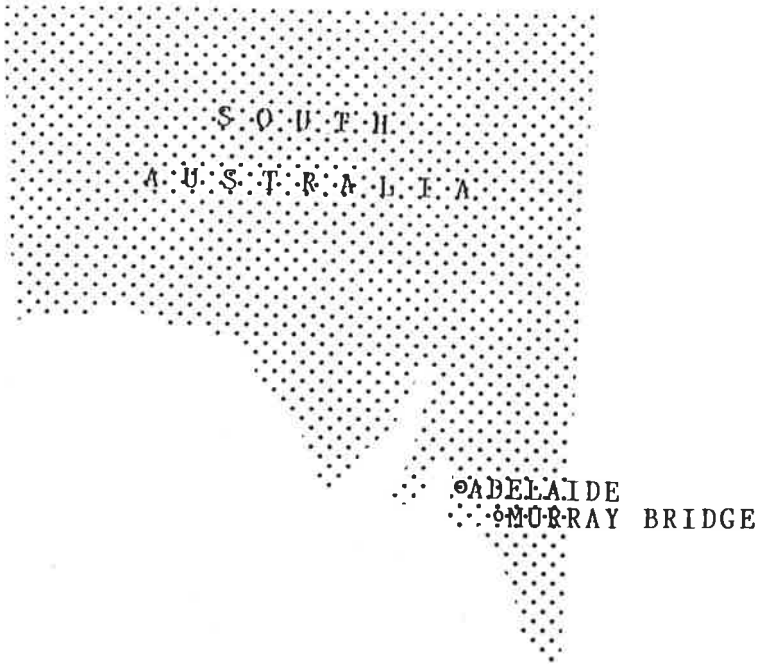
The predominant rotation in the district is wheat-pasture-pasture/fallow. Occasionally, a barley crop is sown after the wheat crop. The length of fallow in the third year varies, ranging from initial cultivation in late July to early October, with seeding in May or June of the following year.

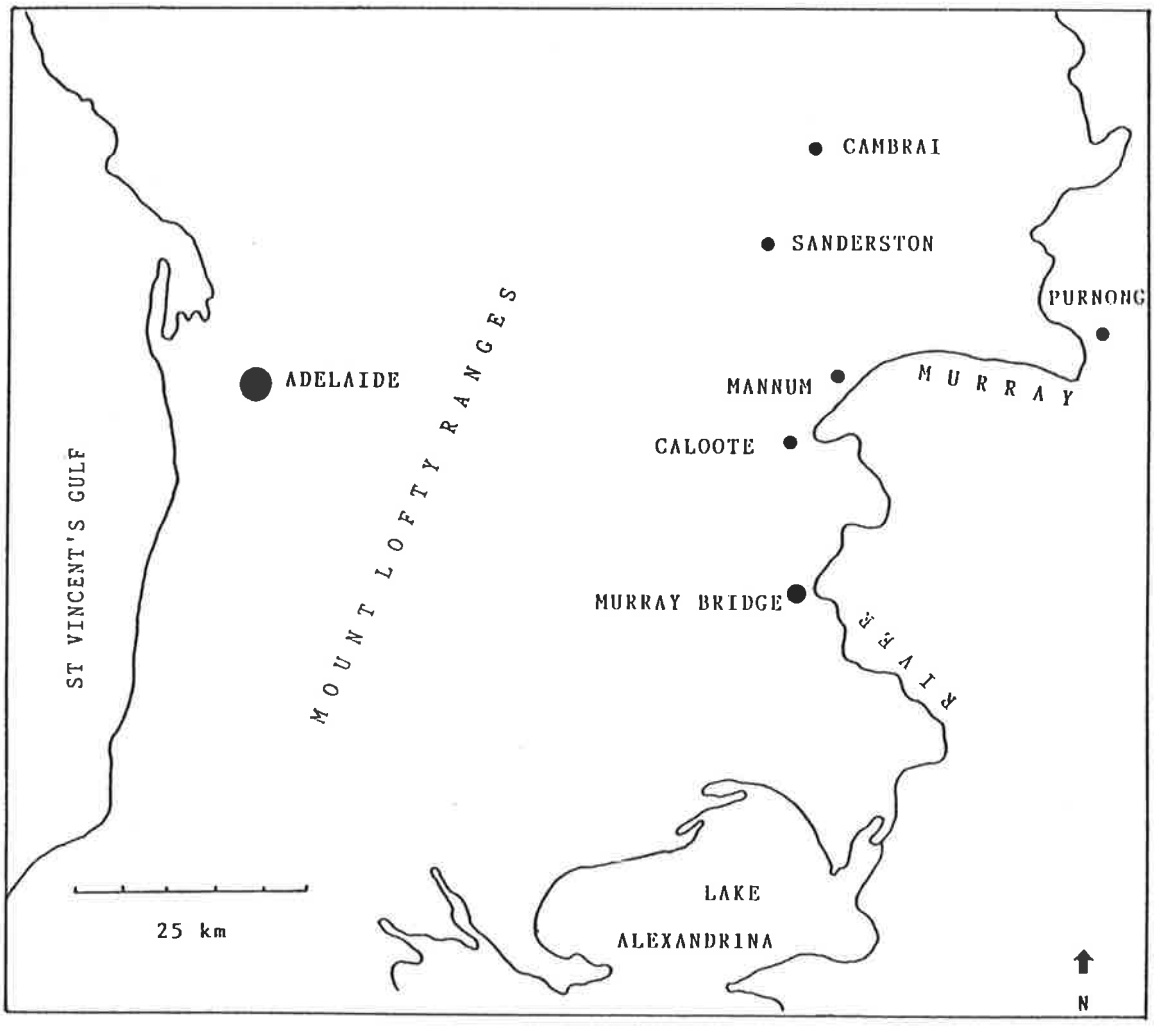
Soil Type

The landscape in the Murray Mallee region is an undulating plain with small areas of isolated sand dunes (Laut *et al.*, 1977). The predominant soil types in the area are calcareous earths. Inherently, these soils are of low fertility, with very low phosphorus and nitrogen contents, and some trace element deficiencies (particularly zinc). However, regular applications of phosphorus and the cultivation of leguminous pastures have raised the fertility of these soils.

Calcareous earths (Gc 1.1 and 1.2; Northcote *et al.*, 1975) are typified by the brownish sandy and loamy mallee soils, which are calcareous throughout the profile.

FIGURE 3.1: Location of field sites in the Murray Mallee region of South Australia. Mannum: latitude 34° 55' S, longitude 139° 18' E.


S O U T H
A U S T R A L I A
ADELAIDE
MURRAY BRIDGE


CAMBRAI
SANDERSTON
PURNONG
ADELAIDE
MANNUM
M U R R A Y
CALOOTE
MURRAY BRIDGE
R I V E R
ST VINCENT'S GULF
M O U N T L O F T Y R A N G E S
25 km
LAKE
ALEXANDRINA
N

These soils often have indistinct horizons, but at field sites used in this study, there was a distinct boundary between the A and B horizons. The surface soil (pH 7.6 - 9.0) is loamy sand or sandy loam, and the sub-soil (pH 8.5 - 9.5) is sandy clay loam (Northcote *et al.*, 1975). Carbonate concentrations increase with depth in the brown to red-brown A horizon. The light brown B horizon consists of sandy clay loam to light or medium clay.

Rainfall

The monthly and total annual rainfall recorded at Mannum in 1987, 1988 and 1989 is presented in Table 3.1, as is the average annual rainfall.

TABLE 3.1: Monthly and total rainfall (mm) recorded at Mannum [latitude 34° 55' S, longitude 139° 18' E], South Australia in 1987, 1988 and 1989. Source: Bureau of Meteorology, Kent Town, South Australia.

	1987	**1988**	**1989**
January	33	24	5
February	10	2	6
March	12	7	4
April	10	36	7
May	57	47	87
June	37	60	60
July	15	25	39
August	23	17	27
September	10	20	31
October	31	3	14
November	1	32	21
December	89	40	36
Annual Total	328	313	337
Mean Annual Total (1876 - 1989)		296	

Sowing and Maintenance of Field Plots

Cultivation - Areas where plots were sown were subject to the usual cultivation

practices of the farmer concerned. Generally, paddocks to be cropped are fallowed in September-October, then re-cultivated after rain in the summer-autumn period. Prior to seeding in May-June, paddocks are cultivated again, and herbicide is usually applied for weed control. The maximum depth of cultivation is approximately 10.0 cm, and seed is sown to a depth of 5.0 - 7.5 cm.

Fertiliser - Topfos® double strength superphosphate was applied at the rate of 100.0 kg/ha with the seed at the time of sowing.

Seeding Rate - Plots were sown at the rate of 10.0 g of seed for each 1.2 m long plot and 30.0 g for the 4.2 m plots. This was equivalent to a rate of 60.0 kg/ha.

Plot Size - Plots were 0.6 m wide and consisted of four rows spaced at 15.0 cm. The distance between rows of adjacent plots was 30.0 cm. In 1987, 4.2 m long plots were planted at both sites, and 1.2 m long plots in 1988 and 1989.

Herbicide Application - Weeds were controlled, where required, by the application of Hoegrass® (diclofop-methyl, 375 g a.i./l) at 1.0 l/ha for ryegrass (*Lolium* spp.) or 1.5 l/ha for wild oats (*Avena fatua*), and Buctril M® (bromoxynil, 200 g a.i./l) at the rate of 1.0 to 1.4 l/ha for broad leaf weeds. Alternatively, Ally® (metsulfuron-methyl, 600 g a.i./kg) at 7.0 g/ha or Ally® plus MCPA (4-chloro-2-methyl-phenoxy-acetic acid) at 6.0 g Ally® + 100.0 ml MCPA/ha were used to control broad leaf weeds. Pathways of 1.8 m between plots were sprayed out, with herbicide.

Time of Sowing - In 1987, plots were sown at Mannum and Sanderston on June 29. The following year, plots at the Mannum site were sown on June 3, and at the Sanderston site on June 8. Plots at both Mannum and Caloote were sown on June 1 in 1989.

Plots of Machete wheat were included in all field experiments as borders, and also as buffers between treated and untreated areas to eliminate the risk of fumigant affecting adjacent non-fumigated plots.

Fumigation - 1987

Vertafume® soil fumigant, containing 850 g/l methyl bromide (monobromomethane), was applied on June 4 at the rate of 1.0 kg/10 m^2. Fumigant was

administered via a tractor-drawn rig, through five tynes spaced at 24.0 cm, to a depth of 12.0 cm. Fumigant applied to this depth penetrates to at least 31.0 cm (Warcup, 1976), but better penetration may have been achieved in the sandy soils of the Murray Mallee. Fumigant is well dispersed in soils with low organic and clay contents (Raski *et al.*, 1983), although sub-soils at experimental sites do contain a reasonable amount of clay.

A bar behind the rig assisted in sealing the soil surface, and the treated area was immediately covered with clear polyethylene sheeting buried in trenches at the boundaries of the treated areas. Plastic covering maintains lethal concentrations of fumigant at the soil surface where crop debris and other organic matter harbours high populations of pathogenic fungi. Warcup (1976) found that 99.9 - 100% of fungal inoculum was eliminated in soil covered with plastic, and 97 - 99% in uncovered soil. Plastic sheeting remained in place for six days following fumigation, and a further nineteen days elapsed prior to seeding.

Fumigation - 1988 & 1989

Crop King® soil fumigant (98% methyl bromide + 2% chloropicrin) was applied at the rate of 1.0 kg/10 m^2. Areas to be fumigated were covered with clear polyethylene sheeting, which was buried in trenches at the boundaries of each area to be treated (Plate 3.1A). Fumigant was then released under the plastic by puncturing sealed cans of the chemical. After five days, the plastic was removed, and another two weeks elapsed prior to seeding.

Inoculation with Microdochium bolleyi and Bipolaris sorokiniana

M. bolleyi isolate #3071 and *B. sorokiniana* isolate #3061 were grown on PDA plates for ten days. Both had been recently re-isolated from inoculated wheat plants. Ten colonies of each were cut into 1.0 cm^2 segments, and mixed thoroughly with sterile 0.1% NaCl on a magnetic stirrer to disperse spores. Granules of attapulgite (Mallina Holdings Ltd., Claremont, Western Australia; Grade 16 - 30), an inert clay material capable of 100% absorption, were used as carriers for the fungal spores. Spore suspensions were thoroughly mixed with the appropriate weight of granules until all granules were wet.

These were then spread on trays and dried at 40°C in a forced-air dehydrator, mixed regularly to facilitate even drying, and removed only when all granules were dry. *M. bolleyi* + *B. sorokiniana* inoculum consisted of granules coated with a mixture of equal volumes of spore suspensions of the two fungi.

The viability of spores and their adherence to granules was tested. Granules were placed on isolation medium (RA), fifteen per Petri dish, and incubated at 20°C for three to four days. The number of *M. bolleyi* and *B. sorokiniana* colonies per plate could then be recorded. Ten such plates were prepared for each of the three inocula. *M. bolleyi* spores adhered to 100% (±0%) of the granules, whether alone or in conjunction with *B. sorokiniana*. Spores of *B. sorokiniana* adhered to only 55% (±17%) of granules when used alone, and 56% (±14%) when in conjunction with *M. bolleyi*. This information was used to determine the amount of inoculum required to inoculate plots at the appropriate levels.

In 1988, the equivalent of 100 propagules of *B. sorokiniana* was added to each gram of soil per plot row, in the immediate vicinity of the seed. This calculation was based on the assumption that the germinating seed occupies a volume of 1.0 cm^3, and multiplied by the row length to determine the volume of soil per plot row. *M. bolleyi* was added at the rate of 200 propagules/gram of soil, as this species adhered to twice as many granules as *B. sorokiniana* and naturally occurs at higher levels in the soil, at least at field sites used in this study. Plots were inoculated with 70.0 g of the appropriate inoculum, 17.5 g/1.2 m plot row.

Plots were inoculated by hand immediately following seeding. Seed was left uncovered in the furrows and inoculum added in the furrow above the seed (Plate 3.1B). Using rakes, seed was then covered over with soil. This resulted in the majority of inoculum resting above the seed, and some falling into the furrow below the seed.

In 1989, inoculum was prepared on attapulgite granules in the manner described previously. *B. sorokiniana* was applied at the rate of 200 propagules/gram of soil in the plot row in the immediate vicinity of the seed, and *M. bolleyi* at 200 and 400 propagules/gram. Both levels of *M. bolleyi* inoculum were applied alone and in combination with *B. sorokiniana*.

PLATE 3.1: **A.** Areas to be treated with methyl bromide soil fumigant were covered with clear polyethylene sheeting, the edges of which were buried in trenches at the perimeter of the plot area to be fumigated. Fumigant was then released under the polyethylene.

B. In 1988 and 1989, field plots were inoculated with *Microdochium bolleyi*, *Bipolaris sorokiniana*, *M. bolleyi* + *B. sorokiniana* or neither species of fungus. Attapulgite granules coated with spores were administered, by hand, to field plots. Inoculum was spread along the furrows, above the seed, then covered over by raking the ridge of the furrow (➡) into the seed row.

Plate 3.1

A

B

Sampling

At all sample dates, ten plants were dug from each plot. Plants were placed in plastic bags, and stored at 5°C until processing. Soil was washed from the roots under running tapwater, and plants again stored at 5°C in plastic bags. Twelve root segments 1.0 cm long were removed at random from each sample, and stored in vials of SDW at 5°C until plating out on isolation medium. Subcrown internodes were rated for severity and frequency of lesions, and stored in vials of SDW at 5°C until plating on isolation medium.

Shoots from each plot were dried at 80°C in a forced-air dehydrator for at least 48 hours prior to being weighed. Root weights could not be determined for field samples, as it was impossible to extract entire root systems from the soil, especially as plants matured.

3.3 GENERAL MEDIA AND METHODS

Isolation of Fungi

Root and subcrown internode samples were surface-sterilised in 2.5% NaOCl for 60 seconds, then washed in three changes of SDW, prior to plating out on isolation medium. The isolation medium (RA) of Harris and Moen (1985a) was used for all general isolations of fungi from plant material. This medium consisted of half strength PDA, which was autoclaved at 121°C for twenty minutes. The medium was cooled to approximately 55°C, and the following concentrations of antibiotics added prior to pouring:

Streptomycin Sulphate	50 ppm
Neomycin Sulphate	50 ppm
Chloramphenicol	250 ppm.

Plates were incubated at 20 - 25°C for four to five days, then placed under a light bank at room temperature to induce sporulation of isolates. Resulting colonies on each plate could then be identified, and isolation frequencies recorded.

Isolates were identified, directly from the isolation plates, on the basis of colony

form and colour. Spores were examined microscopically as an aid to identification. Occasionally, isolates needed to be transferred to fresh PDA plates to induce sporulation, or to allow identification of slow-growing isolates that were in danger of becoming obscured by those with a faster growth rate.

Disease Rating

A disease rating (DR) for *Bipolaris sorokiniana* infection was calculated, for each replicate, on the basis of severity and incidence of subcrown internode lesioning. Subcrown internodes from each sample were divided into four categories, and each category assigned a value (Russell and Sallans, 1940; Tinline *et al.*, 1975; Tinline and Ledingham, 1979):

(1) Clean (0% of subcrown internode lesioned), value = 0

(2) Slight (1 - 25% of subcrown internode lesioned), value = 1

(3) Moderate (25 - 50% of subcrown internode lesioned), value = 2

(4) Severe (>50% of subcrown internode lesioned), value = 4.

The following formula was used to calculate the disease rating as a percentage (Tinline *et al.*, 1975; Tinline and Ledingham, 1979):

$$\frac{\Sigma \text{ (category value x number of subcrown internodes in category)}}{\text{total number of subcrown internodes in sample x 4}} \times 100$$

Determination of Bipolaris sorokiniana Spore Numbers in the Soil

The isolation medium of Dodman and Reinke (1982) was used to estimate populations of *B. sorokiniana* spores in soil samples from field sites:

Soluble Starch	10.0 g
$NaNO_3$	3.0 g
K_2HPO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4.7H_2O$	50.0 mg

Difco® Agar	15.0 g
Distilled Water	to 1000 ml.

This medium was autoclaved at 121°C for twenty minutes, cooled to approximately 55°C and then the following antibiotics and fungicides added before pouring into Petri dishes:

Streptomycin Sulphate	100.0 mg
Chlortetracycline HCl	20.0 mg
Kanamycin Sulphate	50.0 mg
Rose Bengal	70.0 mg
[tetraiodotetra-chlorofluorescein sodium salt]	
Benomyl	10.0 mg
[methyl 1-(butyl-carbamoyl) benzimidazol-2-yl carbamate]	
Captafol	1.0 mg
[N-(1,1,2,2-tetrachloro-ethylthio) cyclohex-4-ene-1,2-dicarboximide]	
Dichloran	3.0 mg.
[2,6-dichloro-4-nitroaniline]	

Soil suspensions (1:10) were prepared by adding 10.0 g of oven-dry soil to the appropriate volume of sterile 0.1% NaCl. This suspension was spread evenly over the agar surface, 0.5 ml per plate. Ten replicate plates were prepared for each soil sample. Plates were incubated at 25°C and colonies counted after five to seven days. Resulting colonies were identified microscopically, as species other than *B. sorokiniana* grew on the medium. Other species included *Ulocladium atrum*, *Curvularia inaequalis*, *Alternaria alternata*, *Embellisia chlamydospora* and a *Bipolaris* sp. (probably *B. spicifer*). Colony numbers were then converted to a measure of the number of spores (or colony-forming units) per gram of soil.

Isolation of Pythium spp.: Modified VP3 Medium

The selective medium of Ali-Shtayeh *et al.* (1985), with modifications by Pankhurst and McDonald (1988a), was used to isolate *Pythium* spp. from roots:

Sucrose	20.0 g
$CaCl_2$	10.0 mg

$MgSO_4.7H_2O$	10.0 mg
$ZnCl_2$	1.0 mg
$CuSO_4.5H_2O$	0.02 mg
MoO_3	0.02 mg
$MnCl_2$	0.02 mg
$FeSO_4.7H_2O$	0.02 mg
Thiamine HCl	100 µg
Difco® Cornmeal Agar	17.0 g
Distilled Water	to 1000 ml.

The medium was autoclaved at 121°C for twenty minutes, cooled to approximately 55°C, and the following added:

Pimaricin	5.0 mg
Vancomycin HCl	75.0 mg
Penicillin [benzylpenicillium sodium BP]	50.0 mg
PCNB [pentachloronitrobenzene]	100.0 mg
Rifampicin [3-(4-methylpiperazinyl-iminomethyl) rifamycin SV]	10.0 mg.

Plates were stored in the dark as Pimaricin is light-sensitive.

Roots were not exposed to chemical surface-sterilants when isolating *Pythium* spp., as these treatments drastically reduce the frequencies of these fungi recorded. Bratoloveanu (1985) found that treatment with 5.0% NaOCl resulted in only 25% isolation of *P. irregulare*, and washing roots in 0.1% $AgNO_3$ eliminated *P. irregulare* from subsequent isolations. However, rinsing roots in sterile distilled water gave 100% recovery of this fungus.

Root samples were vigorously washed in three changes of SDW prior to plating on the medium. Six 1.0 cm segments that had been randomly selected were plated per Petri dish. Plates were incubated at 25°C and investigated after 48 hours. The frequency of *Pythium* spp. was then recorded, but plates were incubated a further 24 hours to allow any slower growing *Pythium* spp. colonies to emerge (Ali-Shtayeh *et al.*, 1985). *Pythium*

spp. isolated were not identified to species level.

Liquid Culture of Pythium sp.

A *Pythium* sp. isolated from field samples in 1989 was grown in liquid Czapek-Dox + Yeast Extract medium for use in inoculation experiments. The medium was prepared as follows:

$NaNO_3$	2.0 g
KH_2PO_4	1.0 g
KCl	0.5 g
$MgSO_4.7H_2O$	0.5 g
$FeSO_4.7H_2O$	0.01 g
Yeast Extract	0.5 g
Sucrose	30.0 g
Distilled Water	to 1000 ml.

Containers of sterile medium were inoculated from cultures grown on PDA plates at 25°C. Liquid cultures were placed on an orbital shaker at 25°C, and growth was sufficient for use as inoculum after only three to four days. Cultures were filtered on a Buchner funnel, under suction, then washed several times with SDW. Hyphal mats were then placed in SDW and homogenised using a hand-held food processor at high speed for two to three minutes. This hyphal homogenate was then used to inoculate plants.

Enumeration of Root Lesion Nematodes in Soil and Root Samples

Pratylenchus neglectus were extracted from soil samples of 500 g using a Seinhorst elutriator (Seinhorst, 1956, 1962). The resulting suspension was passed through two sieves with 53 µm openings, and a modified Baermann funnel was used to obtain samples of nematodes free of soil and organic matter. The modified Baermann funnel consisted of a 53 µm sieve (covered in Kleenex® tissue) in a Petri dish of water. Nematodes pass through the sieve into the water, and 24 hours was allowed for this to occur.

Nematodes were also extracted from the roots of plants. Roots were cut into 1.0 cm lengths and spread on a coarse mesh disk that had been wrapped in tissue. This was placed within a plastic funnel and positioned in a mister (Southey, 1986). At intervals of ten minutes, a fine mist of water at 25°C sprayed over the roots for 15 seconds. Over four to five days, water collected in a test tube below each funnel, and nematodes were extracted from this through a grade four sintered glass funnel under suction.

Nematodes extracted from soil or roots were counted microscopically with the aid of a modified Doncaster (1962) counting dish. The perspex counting dish was marked with four concentric circles to aid in counting. Nematode numbers in a known volume of distilled water were determined by counting the number in 1.0 ml, after first mixing the nematode suspension thoroughly.

Nematodes collected in this manner were subsequently used to inoculate plants growing in pots of steam-sterilised soil. Pots and growing conditions were the same as those described for inoculation tests with fungi. In turn, these roots were placed on the mister, and the number of nematodes extracted from the root systems counted as described above.

Staining Nematodes in Roots

Larvae, adults and eggs of *P. neglectus* were detected and counted, microscopically, in cortices of stained roots. Root samples were stained in lactophenol-cotton blue (Southey, 1986), consisting of 0.1% cotton blue (analine blue) in lactophenol:

Phenol Crystals	200.0 g
Lactic Acid	250.0 ml
Glycerol	300.0 ml
Distilled Water	to 1000 ml.

Roots were boiled in the stain for three minutes (in a fume hood), then cleared and stored in fresh lactophenol.

To avoid working with toxic phenol, later root samples were stained with acid fuchsin (Bridge *et al.*, 1982) or cotton blue in lactoglycerol. This consisted of a solution of equal parts glycerol, lactic acid and distilled water, plus 0.05% acid fuchsin or 0.1%

cotton blue. Roots were boiled in the stain for three minutes, cooled, then washed in distilled water. Samples were cleared and stored in 1:1 glycerol and distilled water, acidified with a few drops of lactic acid.

Fixative for Nematodes

Prior to sectioning to detect nematodes, roots were fixed in FAA (formal acetic alcohol) fixative for 24 - 48 hours (Southey, 1986):

95% Ethanol	20.0 ml
Formalin	6.0 ml
Glacial Acetic Acid	1.0 ml
Distilled Water	40.0 ml.

Roots were sectioned by hand, stained in lactophenol-cotton blue, cleared in lactophenol and investigated microscopically for the presence of stained nematode sections within the root.

Data Analysis

All data are presented as mean values of the number of replicates indicated. Instances in which data were not available are represented by "···".

Analyses of variance were performed, and the least significant difference (LSD) calculated at the 95% ($P<0.05$), 99% ($P<0.01$) and 99.9% ($P<0.001$) significance levels. The LSD at 95% only is included in tables of data, unless otherwise indicated. The abbreviation NS indicates no significant difference between the means being compared. Overall treatment or varietal means were calculated where appropriate, particularly in Chapters 4 and 8. "LSD means" indicates the significant difference between these overall means. "LSD treatment(s)" were calculated to detect significant differences between treatments within individual wheat varieties or sites. Similarly, "LSD varieties" apply to the comparison between wheat varieties within individual treatments.

Percentage data (namely, disease ratings and fungal isolation frequencies) were transformed prior to analysis of variance under the following circumstances. When values were between 0% and 30%, with most less than 10%, and especially when there were

many zero values, the $\sqrt{(x+0.5)}$ transformation was used. The $\sqrt{x}$ transformation was carried out on data between 0% and 30% when fewer zero values existed, and most were greater than 10%. The arcsine $\sqrt{\%}$ transformation of Bliss (Gomez and Gomez, 1984) was used on percentage data in the range 0% to 100% when zero values were also present. Prior to using the arcsine $\sqrt{\%}$ transformation, 0% values were converted to $(^{n}/_{4})$ and 100% values to $(100-^{n}/_{4})$, where "n" is the number of units upon which percentage data were based. Percentage data without zero values were analysed without transformation.

3.4 ABBREVIATIONS

DR	disease rating
PDA	potato dextrose agar
RA	general isolation medium
SDW	sterile distilled water
TWA	tap water agar
VP_3	*Pythium* isolation medium

CHAPTER 4

FREQUENCY OF FUNGI INFECTING WHEAT ROOTS IN THE FIELD

4.1 INTRODUCTION

Wheat roots suffer considerable damage, but the cause of this damage does not seem to be explained by current hypotheses (Chapter 1). Rovira (1987) considers *Gaeumannomyces graminis*, *Rhizoctonia solani* and cereal cyst nematode to be major causes of cereal root disease in southern Australia, and ranks *Bipolaris sorokiniana* and *Fusarium* spp. as pathogens of only minor importance. However, *B. sorokiniana*, alone or in conjunction with other fungi, may be responsible for the observed root damage. This hypothesis was tested, in the field, in 1987, and the species of fungi most frequently associated with diseased wheat roots were determined.

Wheat varieties differing in their reaction to *B. sorokiniana* were sown at two sites in the Murray Mallee (Figure 3.1), on the properties of B. Ramm (Mannum) and K. Maxwell (Sanderston). Plots were sown on areas treated with methyl bromide soil fumigant (as described in the General Methods), and on adjacent untreated areas. The occurrence of *B. sorokiniana*, *Fusarium* spp., *Microdochium bolleyi* and *G. graminis* infecting roots and subcrown internodes was assessed, and their relationship to poor plant growth examined.

The pathogenicity of *B. sorokiniana* to wheat is well established, although the frequency and severity of disease caused by this species varies regionally (Mathre, 1982; Hill *et al.*, 1983) and seasonally (Rovira, 1980). Numerous other species of fungi have been identified in mixed infections with *B. sorokiniana* on cereal roots in South Australia (Moen and Harris, 1980; Harris, 1986), with the *Fusarium* spp. occurring frequently (Fedel-Moen and Harris, 1987). Harris (1987) believes that "minor" pathogens, like *M. bolleyi*, cause more economic crop loss than the so-called "major" pathogens, especially under conditions of host plant stress. Mixed infections of these fungi have generally been overlooked as a cause of cereal root disease.

Soil Fumigation

Soil fumigation is uneconomical for use in broadacre agriculture in Australia (Sivasithamparam *et al.*, 1987), but is an invaluable experimental technique used to demonstrate the potential of crops grown on soils with reduced populations of pathogens, provided the effects of fumigation on factors other than pathogen incidence are also considered.

Russell and Hutchinson (1909) were the first to demonstrate that fumigation could increase the yield of cereals. They achieved 20 - 50% yield increases in plants grown on soil that had been treated with toluene. These yield increases were attributed to enhanced bacterial activity in treated soil and the consequent increase in mineralisation of plant nutrients. The importance of soil-borne pathogens was only realised later (Salt, 1971). Much, if not all, increased growth under soil fumigation is due to healthy root systems capable of more efficient nutrient (Anon., 1987) and water uptake.

Soil fumigants eliminate or reduce populations of fungi, bacteria, weeds, nematodes, mites and insects. Harmful as well as beneficial organisms are killed, and fumigants can interfere with organisms that normally keep soil-borne pathogens in check (Williams and Salt, 1970). However, Ridge and Theodorou (1972) found that populations of aerobic bacteria in fumigated soil originally declined, but then rapidly increased to ten times the population in untreated soil. Fluorescent pseudomonads also decreased in frequency following fumigation, but populations then increased, and these organisms eventually constituted 78% of the total aerobic microflora. After fumigation, a large proportion of the fluorescent pseudomonads from the rhizosphere of wheat plants were found to be antagonistic to *G. graminis* (Ridge, 1976). These may control *G. graminis* not directly killed by fumigation, or that which re-colonises treated soil.

Some organisms produce structures that are unharmed by fumigation. For example, the thick-walled oospores of *Pythium* spp. are not affected by methyl bromide (Stasz and Martin, 1988). Warcup (1976) found that some fungi survived fumigation, while others re-colonised treated areas. The surface soil was rapidly re-colonised via air-borne inocula, but the sub-surface soil was re-infected more slowly. Initially, Warcup (1976) isolated fewer fungi from wheat roots growing in fumigated soil than in non-

fumigated soil, but by 61 days after treatment these differences were not evident.

Fumigation can sometimes increase plant growth in the absence of serious root pathogens (Martin, 1963; Jenkinson and Powlson, 1970; Altman, 1970; Williams and Salt, 1970), due to increased availability and uptake of nutrients. A combination of factors, as described by Rovira (1976), is responsible for enhanced plant growth and nutrient uptake following fumigation:

(1) alteration of the soil microflora, including the initial reduction in populations of root pathogenic fungi,

(2) enhanced release of NH_4^+ nitrogen in the soil (suggesting rapid re-establishment of nitrifying organisms in the soil), with a consequent increase in NH_4^+ uptake by plants,

(3) increased concentrations of bicarbonate-extractable phosphate,

(4) increased root growth,

(5) enhanced growth of root hairs, thus greatly increasing phosphate uptake by plants (Barley and Rovira, 1970).

The response to fumigation does not strictly indicate yield potential in the absence of soil-borne pathogens, but may give some estimation of the extent of the problem.

4.2 METHODS

Six wheat varieties, each replicated five times, were sown on both fumigated and untreated areas at each site. Varieties had the following reactions to *B. sorokiniana*:

Kite - moderately resistant

Schomburgk - moderately resistant

Dagger - moderately susceptible

Oxley - moderately susceptible

Machete - susceptible

Miling - susceptible

(J. Lewis and A. J. Rathjen, personal communication; Dubé and Brooks, 1986; Wallwork, 1989; P. J. L. Whittle, personal communication).

Plots consisted of four rows, each 4.2 m long, as described in the General Methods. The fumigation procedure is also described in Chapter 3.

Determination of Bipolaris sorokiniana Spore Populations in the Soil

The density of *B. sorokiniana* spores in the soil at Mannum and Sanderston was determined in June, 1987, prior to seeding. Soil was sampled on a transect across the plot area at each site. Approximately 500 g of soil was collected (from below the surface litter) every 5 - 6 m. The soil sample from each site was mixed thoroughly and a 200 g sub-sample retained for analysis. Ten soil dilution plates were prepared for the sample from each site, following the method of Dodman and Reinke (1982) as outlined in the General Methods.

Resulting fungal colonies were identified by microscopic examination of spores, as fungi other than *B. sorokiniana* grow and sporulate on this "selective" medium. The number of spores per gram of soil was thus determined for *Alternaria alternata*, *Embellisia chlamydospora*, *Ulocladium atrum* and *Curvularia inaequalis* as well as *B. sorokiniana.*

Sampling

Plants were sampled on August 6, August 31 and October 26. Ten plants were dug from each plot, and stored in plastic bags at 5°C until soil was washed from the roots under running tapwater. Subcrown internodes were rated for *B. sorokiniana* infection, based on the incidence and severity of lesions, on August 31 and October 26 (using the technique outlined in the General Methods), and subsequently plated on RA medium to ascertain the identity of fungi within these tissues.

Dry shoot weight per plant was determined on August 31 and October 26 for the five replicates of each variety.

In early December, all plots were harvested. Whole plants were pulled, by hand, from the two centre rows of each plot (which had not been sampled during the season) and the following parameters determined:

total shoot weight per plant

number of plants per 4.2 m plot row

number of fertile tillers per plant

number of sterile tillers per plant.

Tillers were counted on a twenty plant sub-sample from each plot. All plants were then threshed, and the grain yield per plant determined.

Isolation and Identification of Fungi

Roots and subcrown internodes were removed from each set of ten plants and stored in SDW at 5°C until plating. Twelve 1.0 cm seminal root segments, removed at random, from four replicates were plated on isolation medium (RA) at each sample date, and all subcrown internodes from two replicates were plated.

Fungi were identified directly from the isolation plates on the basis of colony form and colour, and by microscopic examination of spore structure. Numerous species other than *G. graminis*, *B. sorokiniana*, *M. bolleyi* and *Fusarium* spp. occurred on isolation plates, although sporadically and at low frequencies. Data for these species are not presented. It was necessary to transfer some isolates to fresh PDA plates for examination and, in some cases, to induce sporulation to allow identification. This was done especially in instances where fast-growing fusaria, *Trichoderma* or *Rhizopus* had obscured the slower growing colonies.

The occurrence of *B. sorokiniana* and *G. graminis* in seminal root and subcrown internode tissues was recorded. The frequency of *M. bolleyi* and *Fusarium* spp. was also determined because these fungi were so abundant at the two field sites. Records of fusaria were not divided into species, but those present were predominantly *F. equiseti*, *F. oxysporum*, *F. acuminatum* and *F. culmorum*. *F. graminearum* was also isolated, but this species was more likely to infect subcrown internodes than seminal roots, and is primarily a pathogen of the crown and stem base, causing "crown rot" without significantly contributing to root rot. *F. equiseti*, *F. oxysporum* and *F. acuminatum* are the species most commonly encountered on cereals in South Australia (Harris, 1986; Fedel-Moen and Harris, 1987), although the constitution of the *Fusarium* microflora can vary regionally and seasonally.

Isolates of *G. graminis* were not identified to variety level. *G. graminis* var. *tritici* infects wheat, but the less pathogenic *G. graminis* var. *graminis* may also be present.

Data for fungal frequencies are presented as mean values over all varieties, effectively increasing replication. There were varietal differences in the frequencies of fungi recorded, but these were not consistent between harvests or sites.

4.3 RESULTS

Density of Bipolaris sorokiniana Spores in the Soil

Spores of *B. sorokiniana* were detected at moderate levels in soil samples from Mannum and Sanderston (Table 4.1). Both sites had a concentration of 140 spores per gram of soil. Other species were also isolated on the soil dilution plates, at much higher frequencies than *B. sorokiniana* at the Sanderston site. All species, except *B. sorokiniana*, occurred at higher levels in the Sanderston than Mannum soil. Only *Curvularia inaequalis* was higher in number than *B. sorokiniana* at Mannum. *Alternaria alternata* and *Ulocladium atrum* were the species most frequently detected at Sanderston.

There was enormous variation in spore numbers between replicate plates, despite having mixed the soil thoroughly. Some plates contained no colonies of *B. sorokiniana*, while others contained the equivalent of up to 800 spores per gram of soil.

TABLE 4.1: Density of fungal spores (spores/gram of soil) detected in soil from Sanderston and Mannum in June, 1987. (Values are the mean of ten replicate soil dilution plates for each site).

	NUMBER OF SPORES/GRAM SOIL	
Species	**Sanderston**	**Mannum**
Bipolaris sorokiniana	140	140
Alternaria alternata	820	20
Embellisia chlamydospora	420	40
Ulocladium atrum	820	20
Curvularia inaequalis	380	220

Effect of Variety on Fungal Frequencies

Varietal effects on fungal frequencies were not consistent between sample dates or sites. Few of these differences in infection levels were statistically significant at Sanderston, or for the samples taken in October. For example, seminal roots of Schomburgk were infected significantly ($P<0.05$) more by *M. bolleyi* than were those of any other variety at both sites on August 6, but by August 31 and October 26, this species colonised seminal roots of Miling and Kite significantly ($P<0.05$) more often than those of most other varieties. However, Machete and Miling (both susceptible to *B. sorokiniana*) did tend to be infected, on some occasions, significantly more by *B. sorokiniana* than did other varieties.

As few conclusions could be drawn from these varietal differences, fungal isolation frequencies are presented as means over all varieties (Tables 4.2a, 4.2b, 4.2c).

Isolation of Fungi

Frequency of *M. bolleyi*, *Fusarium* spp. and *G. graminis* was reduced by fumigation at both sites on August 6 (Table 4.2a), but by October 26 (Table 4.2c) there was little difference between fumigated and untreated plots. *B. sorokiniana* seemed to be little affected by fumigation as its frequency was actually higher on fumigated than on non-fumigated plots at Sanderston on August 6 (Table 4.2a). Frequency of *B. sorokiniana* was reduced by fumigation more at Mannum that at Sanderston, but this species had re-colonised treated areas by October (Table 4.2c), which was particularly obvious in the isolations from subcrown internodes. Similarly, fumigation reduced the incidence of *M. bolleyi* and *Fusarium* spp. on the first sample date (Table 4.2a), more so at Mannum than at Sanderston. These species also re-colonised fumigated areas as the season progressed (Tables 4.2b, 4.2c).

The majority of isolates recorded were either *M. bolleyi* or *Fusarium* spp. In late August (Table 4.2b), *M. bolleyi* was more common than *Fusarium* spp. on the seminal roots and was more frequent than *B. sorokiniana* on the subcrown internodes. By October (Table 4.2c), *Fusarium* spp. were more frequently isolated than *M. bolleyi* on the seminal roots and the fusaria were more common than *B. sorokiniana* on the subcrown

internodes.

G. graminis was isolated infrequently, whether the soil had been fumigated or not. By October (Table 4.2c), this species was rare at Sanderston and was not detected at Mannum.

TABLES 4.2a - 4.2c: Frequency of fungi (% plant segments infected) isolated from seminal roots (SRT) and subcrown internodes (SCI) of wheat on fumigated and non-fumigated plots at Mannum and Sanderston. (Values are the mean over all varieties; seminal roots from 24 replicates; subcrown internodes from twelve replicates). Species of fungi recorded were: *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs), *Fusarium* spp. (Fus) and *Gaeumannomyces graminis* (Gg).

TABLE 4.2a: Frequency of fungi (% plant segments infected) on August 6, 1987.

	FUMIGATED				NON-FUMIGATED			
	Mb	Bs	Fus	Gg	Mb	Bs	Fus	Gg
MANNUM								
SRT	2.1	0.7	3.5	0	33.5	8.7	40.5	2.9
SCI	0	0	7.5	0	11.4	10.6	5.8	0
SANDERSTON								
SRT	10.6	8.9	4.5	0.7	22.6	2.8	16.7	2.8
SCI	1.2	2.9	0	0	2.1	6.3	4.0	0

TABLE 4.2b: Frequency of fungi (% plant segments infected) on August 31, 1987.

	FUMIGATED				NON-FUMIGATED			
	Mb	Bs	Fus	Gg	Mb	Bs	Fus	Gg
MANNUM								
SRT	6.3	0	10.8	0	30.7	10.6	23.0	3.3
SCI	14.9	0	20.6	0	16.5	5.8	13.8	0
SANDERSTON								
SRT	10.4	1.3	3.5	0	22.0	2.9	6.5	18.2
SCI	11.1	10.4	7.0	0	14.9	12.1	7.5	1.4

TABLE 4.2c: Frequency of fungi (% plant segments infected) on October 26, 1987.

	FUMIGATED				NON-FUMIGATED			
	Mb	Bs	Fus	Gg	Mb	Bs	Fus	Gg
MANNUM								
SRT	19.3	2.8	41.2	0	15.6	12.1	77.5	0
SCI	13.8	18.8	62.5	0	7.9	18.5	77.4	0
SANDERSTON								
SRT	23.5	5.8	15.8	1.4	30.5	13.4	52.8	2.4
SCI	7.8	15.0	21.4	0	20.0	13.8	64.6	0

Other Species of Fungi Isolated

Numerous species of fungi besides *M. bolleyi*, *Fusarium* spp., *B. sorokiniana* and *G. graminis* were isolated from subcrown internodes and seminal roots. These occurred sporadically and at low frequencies, so data concerning their occurrence are not presented. Species included those that Harris (1986) and J. R. Harris and R. Moen (personal communication) have recorded in mixed infections on cereal roots in South Australia: *Curvularia inaequalis*, *Ulocladium atrum*, *Periconia macrospinosa*, *Embellisia chlamydospora*, *Alternaria alternata*, *Cladosporium* sp., *Phoma terrestris* and *Trichoderma* sp. Isolates of *Penicillium*, *Aspergillus* and *Rhizopus* were also recorded.

Shoot Dry Weight

The growth response of wheat to methyl bromide soil fumigant is demonstrated in Plate 4.1. Fumigation significantly ($P<0.05$) enhanced shoot weight of all varieties at both sites on August 31 (Table 4.3a). However, by October (Table 4.3b) and December (Table 4.3c), few of these increases in plant weight due to fumigation were significant for individual varieties. Overall, fumigation increased shoot weight by 67% at Mannum and by 38% at Sanderston in August (Table 4.3a). The corresponding increases were, at Mannum and Sanderston respectively, 24% and 28% in October (Table 4.3b), and 48% and 25% by December (Table 4.3c). Excepting the difference at Mannum in October,

PLATE 4.1: Growth response of wheat to methyl bromide soil fumigant. Plots in the foreground are growing on untreated soil, and those in the background are on areas treated with fumigant.

Plate 4.1

these were all significant (P<0.05).

TABLES 4.3a - 4.3c: Dry shoot weight of wheat varieties on fumigated (Fum) and non-fumigated (NF) plots at Mannum and Sanderston. (Values are the mean of five replicates for each variety).

TABLE 4.3a: Dry shoot weight (mg/plant) on August 31, 1987.

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	598.0	180.5	523.2	304.2
Schomburgk	543.6	214.0	580.5	333.6
Dagger	552.5	152.2	436.9	268.5
Oxley	527.5	178.7	436.6	315.3
Machete	645.8	225.4	583.0	379.8
Miling	647.3	198.7	494.0	294.6
Mean	585.8	191.6	509.0	316.0
LSD(0.05) Varieties	104.9	108.7	116.7	78.3
LSD(0.05) Treatments	153.0		104.7	
LSD(0.05) Means	48.0		45.7	

TABLE 4.3b: Dry shoot weight (g/plant) on October 26, 1987.

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	4.7	2.2	4.4	3.4
Schomburgk	3.3	3.3	4.8	3.2
Dagger	2.7	1.8	4.0	3.0
Oxley	2.1	2.0	4.2	2.9
Machete	2.5	2.9	4.7	3.0
Miling	3.6	2.0	5.0	3.9
Mean	3.2	2.4	4.5	3.2
LSD(0.05) Varieties	1.5	3.0	1.2	1.3
LSD(0.05) Treatments	2.2		1.3	
LSD(0.05) Means	1.2		0.3	

TABLE 4.3c: Dry shoot weight (g/plant) in December, 1987.

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	5.0	2.0	6.3	5.7
Schomburgk	3.8	2.0	7.6	4.8
Dagger	3.0	1.5	6.0	3.9
Oxley	3.0	1.5	6.1	4.7
Machete	2.5	2.0	5.7	4.5
Miling	4.1	2.2	5.4	4.3
Mean	3.6	1.9	6.2	4.7
LSD(0.05) Varieties	1.7	1.5	1.5	1.4
LSD(0.05) Treatments	1.8		1.5	
LSD(0.05) Means	0.8		0.8	

On the untreated plots, Machete had the highest shoot weight in August, at both Mannum and Sanderston (Table 4.3a). In October, the shoots of Schomburgk at Mannum and Miling at Sanderston had the highest weight on untreated plots, although the differences between varieties were not significant (Table 4.3b). At harvest in December, Miling at Mannum and Kite at Sanderston had the highest shoot weights on non-fumigated plots (Table 4.3c).

Disease Rating

Overall, fumigation did not significantly reduce the disease rating of plants at either site on any sample date. In August, Machete had the highest disease rating on untreated plots at Mannum, and Miling the highest on these plots at Sanderston (Table 4.4a). However, there were no significant differences between varieties on untreated plots at either site.

The disease ratings had increased by October, but differences between varieties on untreated plots were not significant (Table 4.4b). Dagger and Machete had the highest values on untreated plots at Mannum, as did Dagger and Miling at Sanderston.

TABLES 4.4a - 4.4b: Disease rating (%) on subcrown internodes of wheat varieties on fumigated (Fum) and non-fumigated (NF) plots at Mannum and Sanderston. (Values are the mean of five replicates for each variety).

TABLE 4.4a: Disease rating (%) on August 31, 1987.

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	12.8	7.9	4.7	8.1
Schomburgk	7.6	6.9	12.0	19.5
Dagger	3.1	7.6	4.6	8.4
Oxley	3.4	8.5	5.3	4.2
Machete	2.2	25.6	0	0
Miling	3.8	5.7	12.3	21.9
Mean	5.5	10.4	6.5	10.4
LSD(0.05) Varieties	7.0	26.4	15.9	24.5
LSD(0.05) Treatments	30.8		16.3	
LSD(0.05) Means	10.3		4.4	

TABLE 4.4b: Disease rating (%) on October 26, 1987.

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	38.6	23.3	23.0	22.5
Schomburgk	68.3	20.8	42.1	38.5
Dagger	26.7	53.5	42.5	49.3
Oxley	49.6	22.5	33.0	32.7
Machete	25.3	49.3	24.7	17.4
Miling	27.4	37.9	37.6	45.1
Mean	39.3	34.6	33.8	34.3
LSD(0.05) Varieties	23.7	55.1	31.4	39.1
LSD(0.05) Treatments	39.4		30.9	
LSD(0.05) Means	31.5		6.1	

Number of Fertile Tillers Per Plant

Plants produced 38% and 19% more fertile tillers on fumigated than on non-fumigated plots at Mannum and Sanderston respectively, but this difference was not significant for all varieties (Table 4.5). The difference between overall means, however, was significant ($P<0.05$) at both sites. On untreated plots at Mannum, Kite, Machete, Miling and Schomburgk had more fertile tillers than other varieties, but this difference was not significant. Schomburgk had more fertile tillers than other varieties on non-fumigated plots at Sanderston, which was significantly ($P<0.05$) higher than that of Kite and Dagger.

Number of Sterile Tillers Per Plant

Plants actually produced more sterile tillers on fumigated than on unfumigated plots, although this difference was not always significant (Table 4.6). The difference between overall means was, however, significant ($P<0.05$) at both sites. At Mannum, Machete had fewer sterile tillers than other varieties on non-fumigated plots, but varietal differences were not significant. Kite had significantly ($P<0.05$) more sterile tillers than Dagger or Miling on untreated plots at Sanderston.

Number of Plants Per Row

Fumigated plots had 16% and 10% more plants per row than non-fumigated plots at Mannum and Sanderston, respectively, but this difference was not significant for any variety (Table 4.7). The overall means were significantly different at Sanderston but not at Mannum. Oxley and Miling had more plants per row than the other varieties on untreated plots at Mannum, but differences between varieties were not significant. At Sanderston, Dagger and Machete plots had significantly ($P<0.05$) more plants per row than Kite and Schomburgk on untreated areas. On fumigated plots at Mannum, Kite had significantly ($P<0.05$) fewer plants per row than either Dagger or Machete.

TABLE 4.5: Number of fertile tillers per plant for wheat varieties on fumigated (Fum) and non-fumigated (NF) plots at Mannum and Sanderston in December, 1987. (Tillers were counted on a 20 plant sub-sample from each replicate; values are the mean of five replicates for each variety).

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	2.0	1.1	1.3	1.3
Schomburgk	1.8	1.0	2.2	1.6
Dagger	1.4	0.7	1.7	1.2
Oxley	1.6	0.9	1.5	1.4
Machete	1.1	1.1	1.5	1.3
Miling	1.8	1.2	1.8	1.5
Mean	1.6	1.0	1.7	1.4
LSD(0.05) Varieties	0.6	0.7	0.4	0.3
LSD(0.05) Treatments	0.6		0.3	
LSD(0.05) Means	0.3		0.2	

TABLE 4.6: Number of sterile tillers per plant for wheat varieties on fumigated (Fum) and non-fumigated (NF) plots at Mannum and Sanderston in December, 1987. (Tillers were counted on a 20 plant sub-sample from each replicate; values are the mean of five replicates for each variety).

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	0.4	0.3	0.9	0.7
Schomburgk	0.6	0.3	0.7	0.4
Dagger	0.6	0.3	0.6	0.3
Oxley	0.9	0.3	1.1	0.6
Machete	0.4	0.2	0.6	0.5
Miling	0.3	0.4	0.4	0.3
Mean	0.5	0.3	0.7	0.5
LSD(0.05) Varieties	0.3	0.2	0.5	0.4
LSD(0.05) Treatments	0.3		0.4	
LSD(0.05) Means	0.2		0.2	

TABLE 4.7: Number of plants per 4.2 m plot row for wheat varieties on fumigated (Fum) and non-fumigated (NF) plots at Mannum and Sanderston in December, 1987. (Values are the mean of five replicates for each variety).

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	68.8	68.5	76.8	58.2
Schomburgk	78.4	58.7	64.4	58.2
Dagger	111.4	80.3	80.6	78.4
Oxley	100.3	91.8	86.8	74.2
Machete	116.0	77.0	83.0	81.8
Miling	94.8	100.7	68.4	62.8
Mean	95.0	79.5	76.7	68.9
LSD(0.05) Varieties	31.9	58.9	21.7	19.0
LSD(0.05) Treatments	46.5		19.2	
LSD(0.05) Means	18.5		7.0	

Grain Yield

Most varieties had a higher yield on fumigated than on unfumigated plots, although this difference was only significant ($P<0.05$) for Miling and Schomburgk at Sanderston (Table 4.8). There were no significant differences between varieties on untreated plots at either site. However, on non-fumigated plots, Miling had the highest yield at Mannum and Dagger outyielded other varieties at Sanderston. Overall, fumigated plots yielded 21% and 18% higher than non-fumigated plots at Mannum and Sanderston, respectively, but these differences were not significant.

Correlation Between Frequency of Fungi and Plant Growth Parameters

Correlations between the frequency of fungi isolated in October and the parameters determined at harvest in December were calculated over all varieties on non-fumigated plots at both sites (Tables 4.9a, 4.9b). Correlations were also calculated for the disease rating and shoot weight measured in October.

TABLE 4.8: Grain yield (g/plant) of wheat varieties on fumigated (Fum) and non-fumigated (NF) plots at Mannum and Sanderston in December, 1987. (Values are the mean of five replicates for each variety).

	MANNUM		SANDERSTON	
Variety	**Fum**	**NF**	**Fum**	**NF**
Kite	0.9	0.5	0.7	0.7
Schomburgk	0.7	0.6	1.2	0.7
Dagger	0.6	0.4	1.0	0.8
Oxley	0.3	0.4	0.4	0.6
Machete	0.3	0.6	0.6	0.6
Miling	1.1	0.7	1.0	0.7
Mean	0.7	0.5	0.8	0.7
LSD(0.05) Varieties	0.5	0.5	0.3	0.2
LSD(0.05) Treatments	0.6		0.3	
LSD(0.05) Means	0.3		0.3	

Mannum - There was a strong positive correlation between the disease rating in October and the frequency of *M. bolleyi* and *Fusarium* spp. infecting the subcrown internodes (Table 4.9a). Number of plants in December was negatively correlated with the frequency of *M. bolleyi* on seminal roots and with *Fusarium* spp. on the subcrown internodes, but was positively correlated with *B. sorokiniana* on the subcrown internodes and with *Fusarium* spp. on the seminal roots. The number of fertile tillers was negatively correlated with the frequency of *B. sorokiniana* infecting subcrown internodes, and number of sterile tillers was positively correlated with the frequency of *B. sorokiniana* on subcrown internodes in October. There was a weak negative correlation between both plant weight and grain yield in December with the frequency of *B. sorokiniana* infecting subcrown internodes. Shoot weight in October was negatively correlated with the occurrence of *M. bolleyi* on seminal roots and with *B. sorokiniana* on subcrown internodes, but these correlations were only weak. The incidence of *Fusarium* spp. on the seminal roots was positively correlated with shoot weight in both October and December,

and with the number of fertile tillers and the grain yield.

Sandeı ston - Shoot weight in October was negatively correlated with the frequency of *B. sorokiniana* and *M. bolleyi* infecting seminal roots, but was positively correlated with the occurrence of *M. bolleyi* and *B. sorokiniana* on subcrown internodes (Table 4.9b). Disease rating was strongly correlated with the frequency of *Fusarium* spp. on the subcrown internodes. The number of plants per row was negatively correlated with the frequency of *M. bolleyi* on the seminal roots, and the frequency of this species was positively correlated with the number of sterile tillers. However, the frequency of *G. graminis* on seminal roots was positively correlated with the number of plants per row, and the incidence of sterile tillers was negatively correlated with *Fusarium* on the seminal roots. The incidence of *B. sorokiniana* on the subcrown internodes was also positively correlated with the number of sterile tillers, and the number of fertile tillers was negatively correlated with the frequency of this species on seminal roots. Grain yield was negatively correlated with the frequency of *G. graminis* infecting seminal roots (Table 4.9b), although this fungus was isolated infrequently (Tables 4.2a, 4.2b, 4.2c). The frequency of *Fusarium* spp. on seminal roots was positively correlated with yield. Shoot weight recorded in December was negatively correlated with the incidence of *Fusarium* spp. on seminal roots in October, but was positively correlated with the frequency of *M. bolleyi* on seminal roots and with *B. sorokiniana* on subcrown internodes.

TABLES 4.9a - 4.9b: Correlation between frequency of fungi isolated from seminal roots (SRT) and subcrown internodes (SCI) of wheat on non-fumigated plots at Mannum and Sanderston on October 26, and plant growth parameters measured in October and December, 1987. Species of fungi recorded were: *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs), *Fusarium* spp. (Fus) and *Gaeumannomyces graminis* (Gg). DR = disease rating, based on the incidence and severity of subcrown internode lesions. Values were calculated over all varieties and replicates.

TABLE 4.9a: Correlations at Mannum.

	ISOLATIONS OF FUNGI IN OCTOBER							
	Mb		Bs		Fus		Gg	
Plant Growth	SRT	SCI	SRT	SCI	SRT	SCI	SRT	SCI
Shoot Weight (OCTOBER)	-.341	.079	.169	-.372	.864	.210	none	
%DR (OCTOBER)	-.488	.667	.722	.011	-.166	.402	isolated	
Plants/Row (DECEMBER)	-.788	-.211	.130	.452	.447	-.630		
Sterile Tillers (DECEMBER)	-.134	-.797	-.695	.812	-.298	-.834		
Fertile Tillers (DECEMBER)	.246	-.058	-.111	-.520	.726	-.090		
Shoot Weight (DECEMBER)	.208	-.036	-.128	-.436	.676	-.169		
Grain Yield (DECEMBER)	-.001	-.012	-.066	-.435	.704	-.168		

r (5%) = .304; (1%) = .393

TABLE 4.9b: Correlations at Sanderston.

Plant Growth	ISOLATIONS OF FUNGI IN OCTOBER							
	Mb		Bs		Fus		Gg	
	SRT	SCI	SRT	SCI	SRT	SCI	SRT	SCI
Shoot Weight (OCTOBER)	-.390	.678	-.794	.829	-.182	.228	.303	none
%DR (OCTOBER)	.032	.120	-.390	.269	.352	.765	-.302	isolated
Plants/Row (DECEMBER)	-.574	-.269	.102	-.339	-.154	.112	.699	
Sterile Tillers (DECEMBER)	.629	-.353	-.018	.438	-.665	-.073	.400	
Fertile Tillers (DECEMBER)	-.329	.704	-.406	.522	.043	-.224	-.031	
Shoot Weight (DECEMBER)	.838	-.225	.060	.407	-.423	-.183	-.077	
Grain Yield (DECEMBER)	.278	.090	-.080	-.286	.435	.245	-.863	

r (5%) = .288; (1%) = .372

4.3.1 Summary of Results

Field experiments conducted during the 1987 growing season showed that:

(1) *G. graminis* was rarely encountered at either site throughout the growing season.

(2) Frequency of fungi infecting seminal roots and subcrown internodes was reduced by treatment with methyl bromide soil fumigant, but fungi re-colonised treated areas as the season progressed. Plants benefitted from pathogen removal early in the season, when there were significant differences in shoot weights between treatments.

(3) The dominant fungal species in August were *M. bolleyi* and *Fusarium* spp. on seminal roots, and *M. bolleyi* and *B. sorokiniana* on the subcrown internodes. In October, *Fusarium* spp. and *M. bolleyi* were predominant on seminal roots, and *Fusarium* spp. and *B. sorokiniana* on subcrown internodes.

(4) Disease rating was not significantly reduced by fumigation, nor could disease rating be correlated with the incidence of *B. sorokiniana* infecting the subcrown internodes.

(5) Plants from fumigated plots had 19 - 38% more fertile tillers than those from untreated plots, and the overall effect of fumigation on tiller numbers was significant ($P<0.05$).

(6) Fumigated plots had 10 - 16% more plants per plot row than untreated plots, which was significant ($P<0.05$) at Sanderston but not at Mannum.

(7) Plants on fumigated areas yielded 18 - 21% more than those on untreated plots, but the overall yield increase was not significant.

(8) Wheat varieties with moderate resistance to *B. sorokiniana* did not perform any better than the susceptible varieties.

4.4 DISCUSSION

Although it is claimed that *G. graminis* is a major pathogen of wheat in southern Australia (Rovira, 1987), this fungus was isolated rarely from field sites in 1987. *G. graminis*, therefore, cannot be considered to have had a significant impact on the level of root disease at either the Mannum or Sanderston site. On average, less than 3% of roots sampled from non-fumigated plots were infected by *G. graminis*, except at the Sanderston site on August 31, when up to 18% of seminal roots were infected. However, overall infection levels were low and, with the low rainfall in spring, the disease failed to develop. This is often observed in South Australian cereal crops, where *G. graminis* is usually present but not necessarily debilitating (Patel, 1983; Wegener *et al.*, 1989).

In 1980 and 1981, Mayfield (1984) reported that 72 - 98% of South Australian wheat crops were infected with *G. graminis* var. *tritici*, but the disease severity varied markedly between crops. This fungus undoubtedly occurs in South Australian cereal crops every year, but the percentage yield reduction that can be directly attributed to *G. graminis* is quite low. Wegener *et al.* (1989) recorded *G. graminis* in a large proportion of South Australian wheat crops, but only minor yield reductions were detected.

Although yield losses due to *G. graminis* have been estimated at up to 10% (Stynes, 1975; Mayfield 1981), average annual losses are more likely to be in the order of only 1 - 5% (A. J. Rathjen, personal communication).

Maas *et al.* (1989) found that disease rate was linearly correlated with the degree of root colonisation by *G. graminis* var. *tritici*. As *G. graminis* colonised few roots of individual plants, the disease rate at Mannum and Sanderston in 1987 would have been very low, and hence the effect on plant growth and, ultimately, on the grain yield would have been negligible. Rovira (1980) reported that *G. graminis* var. *tritici* was the dominant pathogen of cereals at tillering, and there was a strong negative correlation between the percent of plants infected at tillering and the grain yield. However, even though a large proportion of plants may be infected, this does not indicate that individual root systems are colonised to an extent sufficient to cause yield reductions. At Sanderston, there was a strong negative correlation between the frequency of *G. graminis* infecting seminal roots in October and the grain yield measured in December, even though *G. graminis* infected only 2% of these roots. It cannot be inferred from this correlation that *G. graminis* is solely responsible for diminished yield, and the result suggests that some unidentified factor linked to the occurrence of *G. graminis* in seminal roots is responsible for the yield reduction.

If enough moisture is available, *G. graminis* var. *tritici* infection can spread into the crown and up the culm, resulting in the production of a dark mycelial plate around the stem, beneath the lowest leaf sheath (Butler, 1962; Rovira, 1980; Wiese, 1987). In South Australia, this usually happens in late winter-early spring when conditions are moist and warm. Mycelial plate formation results in plant death ("hay-die"). Alternatively, infection of all or most of the seminal roots below the seed causes severe damage ("take-all"). In the absence of these symptoms, *G. graminis* is probably insignificant as a cause of disease and yield loss. Although there was some rainfall in mid-spring (Table 3.1), neither of these symptoms were observed at Mannum or Sanderston in 1987. Mycelial plate formation did not occur due to the low infection levels and the hot, dry, windy conditions in early and late spring. The "hay-die" phase of the disease was observed at the Waite Institute in 1989, both in the field and on plants grown in large boxes of soil

supplied with adequate moisture. This was devastating, killing nearly all the plants, producing symptoms that were not observed in the field plots in 1987.

"Take-all" was first recognised in South Australia in 1852 (Anon., 1868), and McAlpine (1904) showed that the disease was caused by *G. graminis* (then called *Ophiobolus graminis*). Much time and effort has since been expended on the study of this disease, but there is little quantitative data indicating the extent of yield losses, and the economic importance of this disease still remains in doubt (Wallace, 1987). Overall yield reductions that can be directly attributed to "take-all" are probably quite low. However, "take-all" is recognised as being more severe in years with wet springs, and is often associated with rust epidemics, when seasonal conditions favourable to both fungi occur (A. J. Rathjen, personal communication).

Infrequent isolation of *G. graminis* is unlikely to be an artefact of the isolation technique employed. Maas *et al.* (1989) isolated *G. graminis* var. *tritici* from roots surface-sterilised in 5% NaOCl. Root samples from Mannum and Sanderston were treated with 2.5% NaOCl, which would not have interfered with the subsequent growth of *G. graminis* on agar plates. Maas *et al.* (1989) did, however, find that 1% $AgNO_3$ was a better surface-sterilant than NaOCl when isolating this fungus from root material. Because roots were surface-sterilised, it can be assumed that isolates obtained were growing within the roots, parasitically, and not as saprophytes on the root exterior.

Isolates identified as *G. graminis* had the typical back-curling hyphae described by Walker (1981), but were not identified to variety level. *G. graminis* var. *graminis* and *G. graminis* var. *tritici* are both widely distributed, but *G. graminis* var. *tritici* is the most common variety (Rovira, 1987). *G. graminis* var. *tritici* is more damaging to cereals than is *G. graminis* var. *graminis*. If isolates of both varieties were present, a lower incidence of disease is likely than if only *G. graminis* var. *tritici* infected the plants.

Subcrown internodes were colonised by *M. bolleyi*, *Fusarium* spp. and *B. sorokiniana*. The last species was more likely to infect the subcrown internodes than the seminal roots, and is primarily a pathogen of the subcrown internode. *M. bolleyi* was frequent in August, but by October, *Fusarium* spp. were the dominant colonisers of root and subcrown internode tissues. In South Australia, Fedel-Moen and Harris (1987)

found that *Fusarium* spp. were concentrated on the subcrown internodes, crown roots and stem bases, whereas *B. sorokiniana* occurred principally on subcrown internodes and stem bases. Overall, *Fusarium* spp. were encountered more often than *B. sorokiniana*, which is also supported by the work of Fedel-Moen and Harris (1987) in cereal growing areas of South Australia. "Common root rot" in Queensland is attributed to *B. sorokiniana* (Purss, 1970; Wildermuth, 1986) without notable co-infection by *Fusarium* spp. Early studies in New South Wales, however, emphasised that *F. culmorum* usually accompanied infection by *B. sorokiniana* (Hynes, 1935b, 1937b, 1938). *F. culmorum* is relatively infrequent in South Australia, and its role in root disease in this State is replaced by a mixed infection of *F. equiseti*, *F. oxysporum* and *F. acuminatum* (Fedel-Moen and Harris, 1987).

Sanford and Broadfoot (1934) found that most species of *Fusarium* isolated from wheat in Canada were only weakly pathogenic, but any *Fusarium* spp. isolated from cereal roots should be considered as potentially damaging (R. Moen, personal communication), especially as they often make up such a large proportion of fungal isolates from cereal roots (Gordon, 1954; Hill *et al.*, 1983). *F. equiseti* is only weakly pathogenic to wheat (Bennett, 1935; Johnston and Greaney, 1942; Oswald, 1949), although populations in Nigeria are damaging to wheat (Giha, 1978) and the potential of *F. equiseti* to damage plants may be under-estimated (Joffe and Palti, 1967). Isolates of particular *Fusarium* spp. differ in pathogenicity (Fedel-Moen and Harris, 1987), with at least some isolates of most species pathogenic to cereals (R. Moen, personal communication).

F. graminearum is one of the more pathogenic fusaria. Progressive colonisation of the crown, stem base and lower leaf sheaths results in "crown rot" (Burgess *et al.*, 1981). Proximal regions of seminal and crown roots are also infected, as well as the subcrown internode. However, *F. graminearum* does not cause serious root rot (Burgess *et al.*, 1984), and Liddell (1985) reported that *F. graminearum* did not cause rotting of wheat seminal roots. Conversely, R. Moen (personal communication) found that *F. graminearum* caused cortical discolouration and lesioning of oat seminal roots, and could be devastating to barley seedlings. Her pathogenicity tests demonstrated that *F.*

graminearum inhibited root production of barley to a greater extent than did most other *Fusarium* spp. isolates tested. Unfortunately, R. Moen did not reproduce these tests on wheat.

B. sorokiniana spores occurred at moderate densities in the soil at both Mannum and Sanderston, with 140 spores per gram of soil recorded. However, spore densities determined in this manner are, at best, only a poor estimation of propagule numbers and potential disease incidence. Other fungi were detected on soil dilution plates, at much higher levels than *B. sorokiniana* at the Sanderston site. These species are often associated with plants suffering root rot, but are generally considered to be minor components of the disease complex. Tinline (1984) recorded a range from five to 1105 *B. sorokiniana* spores per gram of soil (with an average of 198 spores per gram) in South Australia. In the Murray Bridge area, where the field experiments reported here were conducted, he found an average of 96 spores per gram of soil, which is in the order of that recorded at Mannum and Sanderston in 1987. In this same area, Tinline (1984) isolated *B. sorokiniana* from 10 - 22% of cereal subcrown internodes, which was similar to the frequency (14 - 18%) recorded at Mannum and Sanderston in October, 1987. Spore numbers can vary considerably between sites. Density of *B. sorokiniana* spores in the soil is similar (79 - 95 spores per gram of soil) in Queensland (Wildermuth, 1986) to that in South Australia, but higher in Canada (Chinn *et al.*, 1962; Chinn, 1965) and Brazil (Diehl *et al.*, 1982), where *B. sorokiniana* causes considerable crop losses.

The moderate spore density was reflected in the low frequency with which *B. sorokiniana* was isolated from subcrown internodes. In Canada, *B. sorokiniana* may be isolated from as many as 80% of subcrown internodes (Sallans and Tinline, 1965; Harding, 1973), but only 6 - 12% of subcrown internodes at 1987 field sites were infected in August, when the disease rating was only 10%. By October, 14 - 18% of subcrown internodes were infected, and the disease rating had increased to 35%. Disease rating (based on the occurrence of dark brown lesions on the subcrown internode) was not, however, correlated with the frequency of *B. sorokiniana* isolated from these tissues, but with the frequency of *M. bolleyi* and *Fusarium* spp. at Mannum and with *Fusarium* spp. at Sanderston. Lesions observed on the subcrown internodes when rating for *B.*

sorokiniana infection may well have been caused by *Fusarium* spp. as they infected 65 - 77% of subcrown internodes in October. In Canada, Harding (1972) also noted that there was poor correlation between disease rating and incidence of *B. sorokiniana* in subcrown internode tissue. *M. bolleyi* infected 8 - 20% of subcrown internodes in October, and may also have contributed to lesioning of these tissues. The low overall frequency of *B. sorokiniana* may have been partly due to the inclusion of two moderately resistant varieties (Kite and Schomburgk) and one moderately susceptible variety (Dagger) in this experiment.

Varieties of wheat, however, with moderate resistance to *B. sorokiniana* did not necessarily perform significantly better than the susceptible varieties. In August, the susceptible variety Machete had a significantly higher shoot weight than Dagger (moderately resistant), but in October and December the moderately resistant varieties Kite and Schomburgk performed significantly better. Although susceptible to *B. sorokiniana*, the disease rating of Machete was 0% at Sanderston in August. The incidence of *B. sorokiniana* infecting subcrown internodes is probably a more reliable guide to disease severity than is the disease rating. Machete and Miling (susceptible) did tend to be infected more by *B. sorokiniana* than other varieties, although not always significantly and not on all sample dates. In contrast, Schomburgk (moderately resistant) had a significantly higher disease rating than other varieties at Mannum in October, and Dagger (moderately resistant) had the highest disease rating at Sanderston. Disease rating of Machete was still lower than that of other varieties in October.

Numerous fungi other than *M. bolleyi*, *Fusarium* spp., *B. sorokiniana* and *G. graminis* were isolated from roots and subcrown internodes throughout the growing season, although sporadically and at low frequencies. These species are often isolated from cereal roots and cultivated soils, but there is little information regarding their pathogenicity to wheat. *Curvularia* spp. have long been associated with root rot of cereals in Australia (Hynes 1935a, 1935b, 1936, 1937b; Millikan, 1942). Although Hynes (1935a, 1936) demonstrated the pathogenicity of *Curvularia* to cereals, it is generally considered a weak pathogen of wheat (Zillinsky, 1983). S. Psalios and R. Moen (personal communication) demonstrated that at least some isolates of *Embellisia*,

Curvularia, *Phoma*, *Periconia* and *Trichoderma* were damaging to oats and barley. These species would probably behave similarly on wheat. *Penicillium* and *Aspergillus* spp. are ubiquitous saprophytes (Domsch *et al.*, 1980). *Rhizopus* spp. are also very common, and can attack some plants (Domsch *et al.*, 1980), but there is no evidence to suggest that they are pathogenic to cereals. *Alternaria alternata*, *Cladosporium* and *Ulocladium atrum* are very common soil-borne fungi, that can also be isolated from plant material, where they grow either parasitically or saprophytically. It is conceivable that at least some of these fungi are components of cereal root disease complexes, contributing to the level of root damage observed in the field.

Fumigation enhanced plant growth and improved root health, while plants on untreated plots suffered considerable root damage. The relationship between frequency of *B. sorokiniana* isolated and poor plant growth was tenuous, especially as spore numbers in the soil and isolation frequencies during the growing season were reasonably low. *G. graminis* was rare, while *Fusarium* spp. and *M. bolleyi* were isolated frequently. The fusaria exacerbate disease caused by *B. sorokiniana*, and *M. bolleyi* may act in a similar capacity in this disease complex.

Shoot growth was enhanced by fumigation during early crop stages, but differences between treatments for individual varieties were often not significant as the season progressed. Overall means were significantly different at Sanderston on the three sample dates, but at Mannum the difference between treatments was only significant in August, prior to the onset of severe water stress. Plants benefitted from pathogen removal early in the season, but fumigated soil was re-colonised by fungi. Fumigation led to the production of more fertile tillers per plant, but all varieties produced more sterile tillers on fumigated than on non-fumigated areas. Plants on fumigated soil grew rapidly, and they tillered, flowered and matured earlier than those on untreated plots. Consequently, these plants were at anthesis during a period of hot, dry, windy conditions, and the tips of almost all heads were shrivelled and devoid of grain, and some were rendered completely sterile. These are classic symptoms of severe water stress. The more luxuriant growth of plants on fumigated plots, and the healthier root systems, appeared to have resulted in a higher transpiration rate, so water availability became the limiting factor in late spring-

early summer. Under these circumstances, sterility of tillers cannot solely be attributed to root disease, although the effect of root rots is exacerbated in hosts stressed by adverse environmental conditions. Nevertheless, fumigated plots produced 18 - 21% more grain than the untreated plots.

Factors other than the elimination or reduction of soil-borne pathogens probably led to some of the enhanced plant growth due to fumigation. It is well known that fumigation results in increased nitrogen availability (Altman and Tsue, 1965; Ebbels, 1969; Jenkinson and Powlson, 1970; Rovira, 1976) and uptake, and can also result in the presence of higher numbers of organisms that are antagonistic to pathogenic fungi. *Trichoderma* spp., which were isolated from Mannum and Sanderston, are often implicated in disease suppression following fumigation (Kreutzer, 1965), and either survive fumigation or rapidly recolonise treated soil (Warcup, 1976). Numbers of aerobic bacteria and fluorescent pseudomonads are depressed by fumigation (Ridge, 1976), but populations soon increase and often exceed those in untreated soil (Ridge, 1976; Ladd *et al.*, 1976; Sivasithamparam *et al.*, 1987). These organisms can be antagonistic to soil-borne fungi that survive fumigation or re-colonise treated soil.

The relationship between fungal isolations and poor plant growth was erratic, although the response to fumigation early in the growing season indicates that soil-borne pathogens are largely responsible for poor plant growth. Unless infection can be correlated with reduced yield and decreased shoot growth, the role of particular fungi in crop loss is doubtful. At the sites investigated in 1987, there were only two significant, negative correlations between fungal frequency in October and yield in December: occurrence of *B. sorokiniana* on subcrown internodes at Mannum, and *G. graminis* on seminal roots at Sanderston. Other indicators of yield (tiller numbers and shoot weight) were variable in their correlation with fungal frequencies. Furthermore, grain yield was limited by water stress, providing no clear evidence that the fungi investigated contributed to yield loss.

The response to fumigation is not adequately explained in terms of reductions in the frequency of the recognised root pathogens. *G. graminis* was rarely isolated, and *B. sorokiniana* infection of subcrown internodes was not correlated with disease rating.

Fusarium spp. and *M. bolleyi* did not appear to contribute to yield loss. These observations lead to two possible explanations. Either fumigation affects some undetected organism (thus improving plant growth), or the damage to roots on untreated plots is due to the combined effects of several organisms. *Fusarium* spp., *M. bolleyi* and *B. sorokiniana* may act in conjunction to cause the observed root damage, and these fungi (especially *Fusarium* spp. and *M. bolleyi*) were the only species isolated at frequencies high enough to be considered potentially damaging.

Although the numerous other species of fungi identified occurred sporadically and at low frequencies, mixed infections of these fungi may cause considerable damage to plants. The incidence of *Rhizoctonia solani*, *Pythium* spp. and nematodes was not investigated, although symptoms of cereal cyst nematode (*Heterodera avenae*) infection were not seen on any plants from either site.

M. bolleyi was frequently detected in seminal roots and subcrown internodes, but the contribution of this species to root disease is uncertain. In view of the apparent infestation of field sites with *M. bolleyi*, and the paucity of information regarding the effect of this species on cereals, further investigations (Chapter 7) into the role of *M. bolleyi* in root disease were conducted.

CHAPTER 5

OCCURRENCE OF *PYTHIUM* SPP. IN THE FIELD

5.1 INTRODUCTION

There are many species of *Pythium* capable of infecting cereal roots, and the role of these fungi in relation to the root damage observed in the Murray Mallee warranted investigation. In 1988, additional plots, treated with phosphorous acid, were included in field experiments sown at the Mannum and Sanderston sites (Figure 3.1), in an attempt to control *Pythium* spp. and examine their role in causing root damage. As described in the General Methods, four varieties of wheat were sown (Oxley, Machete, Dagger and Kite), replicated eight times for each treatment.

Prior to seeding, soil was sampled from the Sanderston site. Machete wheat was planted in pots of this soil, and *Pythium* spp. detected in the roots, although at frequencies lower than those subsequently recorded from field samples during the growing season. Approximately 23% of the root segments from pots were infected by *Pythium* spp. On the basis of these results, it was decided to treat some plots with phosphorous acid in an attempt to control *Pythium* spp. Their role in the root damage observed could then be determined. At the same time, eradication of *Pythium* spp. would enable the effects of other pathogens to be more clearly defined, as *Pythium* spp. may well obscure damage caused by other fungi.

Although not all isolates of pythia are pathogenic, *Pythium* spp. are considered to reduce barley yields in South Australia (Bratoloveanu and Wallace, 1985). *P. irregulare* is common in this State, and is one of the most damaging of the pythia (Sprague, 1950). Pankhurst and McDonald (1988b) found that soil type had a major influence on the ecology and aetiology of *Pythium* spp. populations in the South Australian cereal belt. At Kapunda (red-brown earth; 500 mm annual rainfall) *P. graminicola*, *P. irregulare* and *P. ultimum* dominated the isolations from wheat. Avon ("Mallee soil" - calcareous sandy loam; 350 mm rainfall) had a more diverse pythiaceous flora: *P. acanthicum*, *P.*

irregulare, *P. oligandrum*, *P. violae*, *P. paroecandrum* and *P. vanterpooli*. Propagule numoers in the soil were greater at Kapunda (Pankhurst and McDonald, 1988b) which may, in part, be due to the higher rainfall at this site. Sites in the Murray Mallee where field experiments were conducted have a similar soil type and annual rainfall to Avon. Pittaway and Rathjen (1984) found that *P. graminicola* was frequently isolated from wheat in South Australia, where it played a role in disease development in conjunction with other fungi.

Phosphorous Acid

Phosphorous acid is inexpensive, and its most successful application is in the control of *Phytophthora cinnamomi* root rot of avocado trees (Whiley *et al.*, 1987; Pegg *et al.*, 1987). *Phytophthora* spp. on a wide range of vegetables, flowers, ornamentals, fruit and nut trees, vines and citrus are also controlled by phosphorous acid (UIM Chemicals (Aust.) Pty. Ltd., Industrial and Agricultural Chemists, Queensland). *Pythium* spp. infecting turf can be controlled using phosphorous acid.

Formulations of phosphorous acid are applied as soil drenches, foliar sprays or trunk injections. Solutions of phosphorous acid are adjusted to pH 5.8 by the addition of potassium hydroxide, to form monohydrogen dipotassium phosphite. The active portion (PO_3^-), with fungicidal properties, is transported through the conducting elements of the plant (Whiley *et al.*, 1987). Bacteria and fungi in the soil can oxidise phosphorous acid to phosphate, which has no fungicidal properties (Whiley *et al.*, 1987). The production of phosphate in the soil can lead to enhanced plant growth due to its nutritive effects, thus obscuring any beneficial effects of the chemical in controlling pathogens (P. Gurner, personal communication).

5.2 METHODS

Application of Phosphorous Acid

Phosphorous acid was applied to non-fumigated plots included in 1988 field experiments (Chapter 8). A granular formulation of the chemical was kindly supplied by

AgChem, Pty. Ltd. This was applied at the rate of 5 kg/ha, based on the results achieved in controlling *Phytophthora clandestina* on subterranean clover in Victoria (P. Gurner, personal communication). At the time, no information was available on the use of phosphorous acid in controlling *Pythium* spp. on cereals. Phosphorous acid coated attapulgite granules (the same as those used to inoculate plots with *Microdochium bolleyi* and *Bipolaris sorokiniana*, as outlined in the General Methods) were administered via the cone with the seed at the time of sowing. Eight replicates of each wheat variety were treated in this manner, and eight replicates were treated with methyl bromide soil fumigant as outlined in the General Methods.

Sampling

Frequency of *Pythium* spp. in roots was determined from the phosphorous acid treated plots, fumigated plots and untreated plots. Two replicates of each variety were sampled in July, August and September. Each sample consisted of ten plants. Roots were washed free of soil under running tapwater, and twelve 1.0 cm root segments removed at random from each set of ten plants. Roots were then stored in vials of SDW at 5°C until plating. Seminal roots were plated in July and August, but in September crown roots were sampled because the soil had hardened to such an extent that seminal roots were not easily extracted intact. Modified VP_3 medium (as described in the General Methods) was used to selectively isolate *Pythium* spp. from the roots. Two plates were prepared for each sample, with six 1.0 cm root segments per plate. Frequency of *Pythium* spp. was recorded after incubation at 25°C for 48 hours, and finally after another 24 hours. Isolates of *Pythium* spp. were not identified to species level.

Seedling emergence was recorded at Mannum on June 29, and at Sanderston on July 2. The number of seedlings per 1.2 m plot row was counted in the centre two rows of six replicates of each treatment for the four wheat varieties. Dry shoot weight of plants was recorded in June, July, August, September and November. Six replicates of each variety were harvested on November 18, when whole plants were removed by hand from two 1.2 m long rows per plot. The number of plants per 1.2 m plot row was determined at maturity, as was the number of fertile tillers per plant. Plants were threshed and grain

yield per plant determined.

Since there were few significant differences between varieties, in the variables measured, data presented have been pooled for each treatment. This effectively increases replication.

5.3 RESULTS

Infection of Roots by Pythium spp.

The level of *Pythium* infection was decreased by phosphorous acid treatment at both sites in July and September, but this was not significantly lower than that on the untreated plots (Table 5.1). Fumigation with methyl bromide did not reduce the incidence of *Pythium* infection either. All plots were infected with high levels of *Pythium*, although infection was lower in September than earlier in the growing season.

TABLE 5.1: Frequency (% root segments infected) of *Pythium* spp. isolated from roots of wheat plants at Sanderston and Mannum in July, August and September, 1988. Plots were treated with methyl bromide soil fumigant (F), phosphorous acid (PA) or were untreated (NF). (Values are the mean of eight replicates for each treatment).

	JULY		AUGUST		SEPTEMBER	
Treatment	**Sanderston**	**Mannum**	**Sanderston**	**Mannum**	**Sanderston**	**Mannum**
F	...	...	89.6	85.4	52.1	59.0
NF	93.8	87.5	75.0	89.6	66.7	50.0
PA	81.3	84.4	...	...	62.5	43.8
LSD(0.05)	24.4	18.2	22.9	27.6	19.2	27.4

Seedling Emergence

Fumigated plots at Sanderston had a significantly ($P<0.05$) higher emergence than either phosphorous acid treated or non-fumigated plots (Table 5.2). Emergence on plots

treated with phosphorous acid was marginally higher than that on the non-fumigated plots, but not significantly. There was no significant difference between treatments at Mannum.

TABLE 5.2: Seedling emergence (plants/1.2 m plot row), grain yield (g/plant), number of fertile tillers per plant at maturity and number of plants per 1.2 m plot row in November at Sanderston and Mannum, 1988. Plots were treated with methyl bromide soil fumigant (F), phosphorous acid (PA) or were untreated (NF). (Values are the mean of 24 replicates).

	SANDERSTON				MANNUM			
Treatment	Emergence (plants /row)	Yield (g/plant)	Fertile Tillers /Plant	Plants /Row	Emergence (plants /row)	Yield (g/plant)	Fertile Tillers /Plant	Plants /Row
F	46.8	1.2	2.2	32.9	46.1	1.5	2.1	34.1
NF	36.8	1.2	2.0	33.3	51.6	1.0	1.4	30.2
PA	39.6	1.2	2.0	29.9	45.5	0.9	1.4	29.0
LSD(0.05)	6.1	0.2	0.2	5.7	7.1	0.3	0.2	3.8
LSD(0.05)	Emergence vs. Plants/Row Sanderston = 13.0; Mannum = 11.7							

Grain Yield

At Sanderston, there was no significant difference in grain yield between treatments (Table 5.2). Fumigated plots at Mannum had a significantly ($P<0.05$) higher yield than the plots treated with phosphorous acid or the untreated plots. Treatment with phosphorous acid did not enhance grain yield.

Number of Fertile Tillers per Plant

At both Sanderston and Mannum, fumigated plots produced plants with more fertile tillers than either phosphorous acid treated or non-fumigated plots (Table 5.2).

Number of Plants per Row

At Mannum, plots treated with fumigant had significantly ($P<0.05$) more plants per row than plots treated with phosphorous acid or the untreated plots (Table 5.2). There was no significant difference between treatments at Sanderston.

Plots at Sanderston treated with fumigant had significantly ($P<0.05$) more seedlings per row in July than they did at maturity in November (Table 5.2). At Mannum, all plots had significantly ($P<0.05$) more plants per row in late June than that recorded at maturity. On untreated plots at Mannum, 41% of seedlings died between late June and November, whereas only 26% and 36% died on plots treated with fumigant or phosphorous acid, respectively.

Shoot Dry Weight

Fumigation had a greater effect on plant growth than did phosphorous acid, and growth on phosphorous acid treated plots was not significantly different to that on untreated areas (Table 5.3). At Sanderston, the only significant ($P<0.05$) difference between treatments was in September when plants on fumigated plots had a greater shoot weight than those on plots treated with phosphorous acid. There was no significant difference between treatments at Mannum in June, but in July fumigated plots had a significantly ($P<0.05$) greater shoot weight than other plots. This effect persisted in August, September and November.

Fumigation enhanced shoot growth (Table 5.3), but did not reduce the incidence of *Pythium* spp. isolated from the roots (Table 5.1).

Shoot weight differed between sites, and was higher on untreated plots at Mannum than at Sanderston in June-August, although the reverse was true in September and November (Table 5.3).

TABLE 5.3: Dry shoot weight (mg/plant) of wheat plants at Sanderston (S'ton) and Mannum (M'M) in June, July, August, September and November, 1988. Plots were treated with methyl bromide soil fumigant (F), phosphorous acid (PA) or were untreated (NF). (Values are the mean of four replicates in June, seven in July and August, twelve in September and 24 in November).

	JUNE		JULY		AUGUST		SEPTEMBER		NOVEMBER	
Treatment	**S'ton**	**M'M**	**S'ton**	**M'M**	**S'ton**	**M'M**	**S'ton**	**M'M**	**S'ton**	**M'M**
F	13.5	18.1	45.3	105.7	315.2	524.2	1955.8	2001.7	4673.5	4651.7
NF	14.3	20.6	40.8	64.8	238.5	276.1	1559.8	1070.0	4660.9	2903.9
PA	12.1	20.9	35.6	61.9	...	...	1392.8	906.7	4743.5	2695.2
LSD(0.05)	3.2	4.7	10.1	13.0	96.4	110.8	444.1	432.0	479.8	650.5

5.3.1 Summary of Results

The following results were observed following treatment of field plots with phosphorous acid or methyl bromide in 1988:

(1) Neither phosphorous acid nor methyl bromide soil fumigant significantly decreased levels of *Pythium* infection. Frequency of *Pythium* spp. isolated from the roots was consistently high over all treatments throughout the growing season, although levels of infection had decreased by September when conditions were drier.

(2) Fumigation enhanced seedling emergence, grain yield, number of fertile tillers per plant, number of plants per plot row and shoot dry weight, although not always significantly. Treatment with phosphorous acid did not significantly improve plant growth at either site on any sample date.

(3) Up to 41% of seedlings died between late June and November. This will be discussed in more detail in Chapter 8.

5.4 DISCUSSION

Treatment with phosphorous acid did not control *Pythium* spp. at field sites in

1988. Either the rate of application (5 kg/ha) was too low, or the granular formulation ineffective. Much higher rates of phosphorous acid (up to 100 kg/ha) have also failed to control *Pythium* spp. on cereals (P. T. W. Wong, personal communication). Fenn and Coffey (1984) reported that *Pythium* spp. had a lower *in vitro* sensitivity than *Phytophthora* spp. to phosphorous acid. Even early in the growing season, when the chemical was presumably most active, there was no significant effect on the frequency of *Pythium* spp. isolated from the roots (Table 5.1).

Fumigation with methyl bromide did not significantly affect the frequency of root infection by *Pythium* spp. either. Conflicting reports exist regarding the sensitivity of *Pythium* spp. to methyl bromide. Cook *et al.* (1987) eliminated 95 - 99% of inoculum from the soil with fumigation, increasing wheat yield and plant growth. Growth of sugarcane was enhanced by treating soil with methyl bromide, and only 1% of roots were infected with *Pythium* spp., whereas 24% of roots were infected on untreated areas (Hoy and Schneider, 1988b). Munnecke *et al.* (1978) also considered *Pythium* spp. to be sensitive to methyl bromide.

Pythium spp. produce sporangia, thin-walled oospores and thick-walled oospores. The thick-walled oospores are tolerant of adverse environmental conditions, providing a reservoir of resistant propagules in the soil. Under favourable conditions, these become thin-walled and germinate. Sporangia and thin-walled oospores constitute the infective inoculum. Stasz and Martin (1988) found that the thick-walled oospores of *P. ultimum* were not affected by exposure to methyl bromide or any of five fungicides, nor did this affect their ability to subsequently become infective, thin-walled oospores. They were, however, killed by heat at 70°C, and their viability was reduced by treatment at 50°C. On the other hand, thin-walled oospores and sporangia in a quiescent state were killed by treatment with methyl bromide, heat at 50°C and 70°C, and by all but one of the five fungicides tested. If treated during germination, thin-walled oospores and sporangia were killed by low levels of all the chemicals tested. Stasz and Martin (1988) concluded that the surviving thick-walled oospores replenish the supply of germinable propagules in the soil, re-establishing the potential for disease. Reappearance of *Pythium* spp. in treated soil is therefore due to survival of the thick-walled oospores, rather than re-invasion of

soil subsequent to treatment.

Sensitivity of *Pythium* spp. to methyl bromide may differ between species, but many species exist in the soil, and fumigation at Sanderston and Mannum did not, apparently, eradicate any of them. Reports of effective control of *Pythium* spp. in the field come from regions with different climatic and edaphic conditions to those in South Australia. Surface soil at field sites in the Murray Mallee is sandy, with a low water holding capacity. Summers are hot and dry, so there is no living host material available. Under such adverse conditions, a large proportion of *Pythium* inoculum may exist as thick-walled oospores, and *Pythium* spp. are therefore less vulnerable to chemical soil treatments. Warcup (1976) reported lower levels of *Pythium* infecting wheat roots on fumigated areas than on untreated areas at South Australian field sites, but the fumigant used in his studies also contained a high concentration of chloropicrin.

Very high levels of infection were recorded at both sites in 1988: at Mannum, more than 80% of root segments tested were infected in July and August, and up to 93% of roots were infected at Sanderston. Infection levels were lower in September, when 50 - 60% of roots were infected. *Pythium* spp. require high moisture levels (Domsch *et al.*, 1980), and by September conditions were drier than earlier in the season. Lower temperatures are also more favourable to *Pythium* spp., and September is warmer than the preceding winter months. In Western Australia, Dewan and Sivasithamparam (1988) recorded higher levels of *Pythium* infection on wheat roots at seedling and tillering stages than later during the growing season. They suggest that this is due to the progressive colonisation of roots by other fungi.

Isolates of *Pythium* spp. were not identified to species level, but most were of the "rosette" colony formation, and some displayed the "chrysanthemum" pattern, typical of *P. graminicola* and *P. irregulare*. Bratoloveanu and Wallace (1985) found that, of the eleven species isolated (Bratoloveanu, 1985), the most common pythia in South Australia were *P. graminicola* and *P. irregulare*, both of which they considered to be pathogenic. These were also the most frequent of eight species isolated from wheat in New South Wales (Tesoriero and Wong, 1988). Dewan and Sivasithamparam (1988) isolated seven species of *Pythium* from wheat in Western Australia: three proved to be pathogenic, but

four were not. Chamswarng and Cook (1985) found that all ten species isolated from wheat in Idaho and Washington were pathogenic. The isolation medium used in 1988 was probably not suitable to detect all species of *Pythium*, but gave a useful comparison between treatments, and indicated the high infection levels attained.

Pythium spp. occurred at high levels, but no effective control was achieved to allow the damage caused by these fungi to be demonstrated. On average, 72% of roots from fumigated areas were infected, and 77% from non-fumigated areas. Conversely, Warcup (1976) found that only 54% of wheat roots were infected with *Pythium* spp. following fumigation, whereas 88% of these roots from untreated areas were infected. However, there was still a considerable increase in plant growth due to fumigation in 1988, despite the presence of *Pythium* spp. It can be concluded that the response to fumigation was due to the removal of other soil-borne organisms, of which there are many that infect roots, acting in conjunction to cause the observed damage.

Metalaxyl (Ridomil®) is very effective in controlling pythiaceous fungi (Tesoriero and Wong, 1988). Hoy and Schneider (1988b) reported reduced levels of infection, significant yield increases and significant increases in root and shoot growth when this chemical was used to control *Pythium* on sugarcane. Treatment with this fungicide may have provided more conclusive evidence on the role of *Pythium* spp. in root rot of wheat, but it is poisonous to operators and not favoured as an experimental tool.

CHAPTER 6

INHIBITION OF LATERAL ROOT GROWTH IN THE FIELD

6.1 INTRODUCTION

Samples of wheat and barley plants from various locations in the Murray Mallee (Figure 3.1), and from Mudamuckla and Waramboo on the Eyre Peninsula, exhibited symptoms of root damage that are commonplace in South Australian cereal crops, especially on these lighter soil types. The lateral roots along the length of both seminal and crown roots were severely damaged, and extensive areas of both root systems were devoid of laterals. The crown roots were extensively rotted and decorticated. Roots exhibiting these symptoms were examined to determine the species of fungi responsible for the observed damage, and compared with roots that had healthy laterals. These samples were collected between September and early November of the 1987 and 1988 growing seasons, and represent unreplicated sampling at each of the twelve sites. The root damage observed at these twelve sites was identical to that seen on plants from the more intensively investigated sites in the Murray Mallee.

In 1986, another set of barley samples exhibiting the symptoms described above was collected from M. Kluge's property (Purnong, Murray Mallee; Figure 3.1) at crop maturity in late November. These samples were taken from areas that had undergone solarisation treatment the previous summer, and compared with plants from adjacent untreated areas in the crop.

Soil Solarisation

Solarisation involves covering damp soil with transparent polyethylene sheeting during the summer. This results in elevated soil temperatures, which kill a large proportion of the soil microflora, depending on the temperature attained. Smith *et al.* (1984) recorded a maximum temperature of 48°C at a depth of 15.0 cm in soil covered with polyethylene, whereas the maximum temperature in uncovered soil at this depth was

only 36°C. Katan (1980) recorded similar temperatures: 50°C at 5.0 cm and 44°C at 20.0 cm depth, which was approximately 8 - 12°C higher than in uncovered plots.

Some remarkable yield increases in response to soil solarisation have been reported. Potato yield was increased by 35% due to solarisation of soil infested with *Verticillium dahliae* and the root lesion nematode *Pratylenchus thornei* (Grinstein *et al.*, 1979b). Grinstein *et al.* (1979a) were also able to increase peanut yield by 123% when *Sclerotium rolfsii* was eliminated, and eggplant yield was increased 215% when *V. dahliae* was killed by solarisation (Katan *et al.*, 1976).

6.2 METHODS

Wheat and barley plants were sampled from a total of twelve crops at various locations in the Murray Mallee and on the Eyre Peninsula between September and November of the 1987 and 1988 growing seasons. Barley plants were also sampled from solarised and untreated areas in a crop at Purnong in the Murray Mallee in November, 1986. Each sample consisted of a set of four to six plants.

The area to be solarised was covered with clear polyethylene sheeting in late spring-early summer (November-December), 1985, following a substantial fall of rain. This sheeting remained in place until the autumn of 1986 (March), and the barley crop was sown in May.

Soil was washed from the roots under running tapwater, and symptoms on the roots examined. Twelve 1.0 cm root segments were removed from each set of plants, and plated on RA medium (as described in the General Methods). The species of fungi infecting roots were identified and their frequencies of infection recorded.

6.3 RESULTS

Samples From the Eyre Peninsula and Murray Mallee

Many species of fungi were isolated from the roots of wheat and barley with poor laterals. In most instances lateral roots were produced, but subsequently became rotted

and lesioned, resulting in them appearing as truncated "stumps" or "spikes" along the length of seminal and crown roots. In some cases, extensive areas of the main root axes were devoid of laterals, and the cortex of these axes was degraded. The severity and incidence of symptoms appeared to be greater on the crown roots than on the seminal roots.

Fusarium spp. and *Microdochium bolleyi* were the fungi most frequently isolated from damaged roots, with other species occurring infrequently (Table 6.1). These fungi were also isolated from healthy roots, but *Fusarium* spp. and *M. bolleyi*, respectively, were isolated 55% and 70% more frequently from the diseased than the healthy roots. *Fusarium* spp. and *M. bolleyi* were the only species significantly ($P<0.05$ and $P<0.01$, respectively) more common on diseased than on healthy roots. Other species (*Bipolaris sorokiniana*, *Gaeumannomyces graminis*, *Phoma terrestris*, *Periconia macrospinosa*, *Embellisia chlamydospora* and *Alternaria alternata*) were detected infrequently, and were present at similar levels on samples of both healthy and damaged roots (Table 6.1).

TABLE 6.1: Frequency (% root segments infected) of fungi isolated from wheat and barley roots with healthy or damaged lateral roots, over twelve sites in the Murray Mallee and on the Eyre Peninsula of South Australia in September-November, 1987 and 1988. (Values are the mean over twelve sites). Species of fungi recorded were: Fus - *Fusarium* spp.; Mb - *Microdochium bolleyi*; Bs - *Bipolaris sorokiniana*; Gg - *Gaeumannomyces graminis*; Pht - *Phoma terrestris*; Pm - *Periconia macrospinosa*; Emb - *Embellisia chlamydospora*; Alt - *Alternaria alternata.*

	SPECIES OF FUNGI ISOLATED							
Laterals	**Fus**	**Mb**	**Bs**	**Gg**	**Pht**	**Pm**	**Emb**	**Alt**
Healthy	15.2	7.7	5.8	6.1	3.3	2.6	2.5	0
Damaged	33.8	25.4	4.7	8.5	5.9	5.9	3.0	1.6
LSD	**(0.05)** = 13.3; **(0.01)** = 17.7; **(0.001)** = 22.9							

Samples from Solarised and Non-Solarised Areas

Barley plants exhibited vast differences in growth rate, plant health and yield on solarised compared with untreated areas. The farmer, M. Kluge, estimated a yield of 1.7 - 1.8 tonne/ha as a result of the solarisation treatment, compared to a yield of only 0.7 tonne/ha on the untreated area. Roots from the solarised area suffered less lesioning and grew more extensively through the soil. The most striking difference was in the development of lateral roots along the crown and seminal root axes. When plants were removed from the ground, soil remained attached to the roots from the solarised area, indicating extensive lateral root growth. This was not the case for plants sampled from untreated areas, as soil fell away from the roots. Symptoms on the roots, and damage sustained by the laterals, were identical to those described above for samples collected in 1987 and 1988.

Many species of fungi were isolated from these roots, but few conclusions could be drawn from this due to the late stage of the growing season at which plants were sampled. However, *Fusarium* spp. were isolated from 92% of crown roots from the untreated area, and from 70% on the solarised area. *M. bolleyi* infected 42% of seminal roots from the non-solarised area, but only 11% of roots from the treated area of the barley crop were infected with this species. Other species of fungi isolated included *E. chlamydospora*, *Ulocladium atrum*, *P. macrospinosa*, *Curvularia inaequalis* and *B. sorokiniana*. These were detected sporadically and at low frequencies, whether the soil had been treated or not.

6.3.1 Summary of Results

The following resulted from this investigation into the deterioration of lateral roots:

(1) Lateral roots on seminal and crown root axes of cereal plants suffer extensive damage, which is commonplace in South Australian cereal crops, especially on the lighter soil types.

(2) *Fusarium* spp. and *M. bolleyi* were the species most frequently isolated from

unhealthy roots, and these fungi infected significantly fewer healthy than diseased roots.

(3) Numerous other species of fungi were isolated, but sporadically and at low frequencies.

(4) Soil solarisation reduces the frequency of fungi infecting cereal roots, with enhanced root health and plant growth consequently leading to a higher grain yield.

6.4 DISCUSSION

M. bolleyi can sometimes be associated with damage to lateral roots such as that described here (J. R. Harris, personal communication), although *G. graminis* var. *tritici* has more commonly been identified as the cause of lateral root damage (Deacon, 1981). Highly invasive fungi like *G. graminis* var. *tritici* infect main root axes and lateral roots, causing vascular discolouration at the junction of main roots and their laterals (Deacon, 1976). However, Deacon's (1976, 1981) observations were made in Scotland, where higher water availability would foster the growth of *G. graminis* in soil and roots. In 1987 - 1988, *G. graminis* was isolated infrequently from roots, regardless of the health of laterals.

Fusarium spp. have previously been associated with damage to lateral roots, and appear to be involved in causing the damage observed on field samples collected in 1986 - 1988. The pathogenicity tests of Fedel-Moen and Harris (1987) showed that inoculation with *F. acuminatum* tended to truncate and discolour the lateral roots of barley and oats. R. Moen (personal communication) further demonstrated that various *Fusarium* spp. caused lesioning and discolouration of laterals, and particularly reduced the number of lateral roots on both barley and oats. At least some isolates of each *Fusarium* sp. tested by R. Moen were capable of causing lateral root damage. Unfortunately R. Moen did not test wheat but, as fusaria produce phytotoxins that (among other things) inhibit the development of lateral roots (Katouli and Marchant, 1981), these fungi could have had a similar effect on wheat.

S. Psalios and R. Moen (personal communication) have conducted

comprehensive pathogenicity tests with isolates of numerous fungi that are frequently recovered from the diseased roots of cereals. Many of the species they tested are regarded as "minor" pathogens, but they found that at least some isolates of each species damaged the roots of oats and barley. Isolates of several species (*Phoma sclerotioides*, *Embellisia chlamydospora*, *Curvularia* spp. and *Trichoderma* spp.) damaged the lateral roots of barley, resulting in a decrease in lateral root number or they rendered long sections of the root devoid of laterals. Of the species that S. Psalios and R. Moen tested and found to be damaging to laterals, *E. chlamydospora* was isolated from samples of wheat collected in 1987 and 1988. However, this species colonised roots having healthy or diseased laterals with similar frequencies. *Phoma terrestris* and *Periconia macrospinosa* were also as common on roots with healthy laterals as on those with diseased laterals, and S. Psalios and R. Moen reported no lateral root damage due to these species.

Pythium spp. are frequent invaders of cereal roots, but their occurrence was not assessed on samples in 1987 or 1988. These fungi have occasionally been associated with lateral root damage on wheat and sugarcane. Cook *et al.* (1987) implicated *Pythium* spp. in causing wheat roots to be stripped of root hairs and fine rootlets. *P. arrhenomanes* causes rotting of the lateral roots of sugarcane (Hoy and Schneider, 1988a, 1988b).

Few conclusions could be drawn regarding the species of fungi damaging lateral roots at Purnong in 1986, because samples were taken late in the growing season when it is difficult to ascertain the role fungi have played in causing damage throughout the season, and saprophytes tend to colonise large areas of the senescing roots. Nevertheless, these samples from solarised and untreated areas of a crop were useful in demonstrating two points. Firstly, the damage sustained by cereal lateral roots is extensive. Secondly, soil solarisation indicated that the damage to lateral roots is due to their infection by soil-borne organisms, rather than an effect of the soil structure, water or nutrient status.

Damage to lateral roots must markedly restrict water and nutrient uptake by plants, thus limiting crop yields. This is exacerbated by the concomitant rotting of the root cortex, and consequent loss of root hairs. The progressive destruction of crown root laterals was investigated more fully over the 1989 growing season (Chapter 9), and the organisms responsible were studied in greater detail.

CHAPTER 7

INOCULATION WITH *MICRODOCHIUM BOLLEYI*

7.1 INTRODUCTION

In view of the frequent association of *Microdochium bolleyi* with the diseased roots of wheat in the field (Chapter 4), and its possible role in the rotting and inhibition of lateral root growth (Chapter 6), inoculation experiments were conducted to investigate the effect of this fungus on the growth of wheat, and to determine the specific symptoms associated with infection.

A preliminary experiment (Experiment 1) was conducted with the commercial wheat varieties Miling, Aroona and Kite. Miling often produces shrivelled grain, so was included in this test to determine whether *M. bolleyi* deleteriously affects growth of this variety. A more detailed experiment (Experiment 2) examined the effect of *M. bolleyi* on the breeder's lines (Fund*CP)*MKRK)/8/12, (C8*MM)*MKRK)/18/15 and (C8*MM)*MKRK)/18/22 and the varieties Oxley, Miling and Condor. It had been noticed that these varieties and selections often yielded poorly at some trial sites, even in the absence of recognisable root pathogens. This raised the possibility that some previously unconsidered organism, such as *M. bolleyi*, was causing the yield loss.

7.2 METHODS

Pre-germinated, surface-sterilised seed was planted in pots of steam-sterilised soil, as described in the General Methods for pot experiments. All pots were placed in the glasshouse in a waterbath maintained at 19±2°C.

In the first experiment, three pots of each variety were inoculated with *M. bolleyi*, and three uninoculated pots of each variety included as controls. The second experiment was comprised of three inoculated and two uninoculated pots of each variety and breeder's line.

Inoculation

Three week old colonies of *M. bolleyi,* grown on PDA plates, were cut into 1.0 cm^2 segments and mixed on a magnetic stirrer in 540 ml of sterile 0.1% NaCl to disperse spores. This spore suspension was mixed thoroughly with 1.35 kg of soil, and 420 ml of sterile 0.1% NaCl was mixed with 1.05 kg of soil for the control pots. Steam-sterilised soil (400 g) was added to each pot, and 50 g of soil containing spores (or NaCl for controls) placed above this. Five pre-germinated seeds were placed on the inoculum layer and a further 200 g of steam-sterilised soil added, resulting in a seeding depth of 3.0 cm.

Sampling

Experiment 1: After fourteen days, one inoculated and one control pot of each variety was sampled. Symptoms on the roots were noted and twelve 1.0 cm, randomly selected, seminal root segments plated on isolation medium (RA) for each replicate. Remaining pots were sampled 25 days after planting, and root segments plated on isolation medium in the same manner.

Experiment 2: All pots were washed out 35 days after planting. Symptoms on the roots were observed, and twelve seminal root segments (1.0 cm long) were randomly selected from each replicate for plating on RA medium to determine the incidence of *M. bolleyi* infecting root tissues. Roots and shoots from each replicate were dried and weighed.

7.3 RESULTS

7.3.1 Experiment 1

Symptoms on the Roots

After fourteen days, both inoculated and control plants appeared healthy. Inoculated plants displayed little root damage, and lateral roots and root hairs were well-formed. *M. bolleyi* did not, apparently, interfere with seedling establishment.

Miling - Root systems of inoculated plants were comparable to those of the controls in

both root mass and extent of lateral root formation. Seminal roots of inoculated plants displayed the following symptoms, with only one instance of each:

small, light brown lesion behind root tip

"spiked" tip with associated cortical degradation and stelar browning.

Aroona - Inoculated and uninoculated plants had healthy roots and good lateral root formation. No symptoms were obvious on the roots of inoculated plants.

Kite - All plants had healthy seminal and lateral roots, with no damage evident to inoculated plants.

By 25 days, roots of inoculated plants had suffered more damage than at fourteen days, and a range of symptoms was observed. Roots of Aroona sustained little damage, Miling showed slight lesioning and Kite roots suffered the most lesioning.

Miling - Root mass of inoculated and untreated plants was similar, and all showed extensive lateral root formation. Inoculated plants were generally free of root damage, except one plant which had several light orange-brown, stunted root tips.

Aroona - Although the untreated plants had a larger root mass than those inoculated with *M. bolleyi*, no root damage was evident on the inoculated plants.

Kite - All control plants had healthy lateral roots and a large root mass. Some crown roots were beginning to form on these plants. Roots of inoculated plants, however, sustained some damage and stunting. The following symptoms were observed with only one instance of each:

1.0 cm length of brown cortical discolouration

orange-brown discoloured root on one plant with few seminal or lateral roots

orange-brown root tip.

Re-isolation of Microdochium bolleyi

M. bolleyi was not isolated from lesioned or healthy roots of any variety after only fourteen days, but by 25 days (Table 7.1), *M. bolleyi* was readily re-isolated from the roots of all inoculated plants. The lowest frequency of *M. bolleyi* was recovered from the roots of Aroona, and this variety sustained the least root damage. Of the Miling roots

sampled, 26% were infected, and these roots showed slight lesioning. The highest *M. bolleyi* recovery was from Kite, and the roots of this variety displayed the most serious lesioning and cortical discolouration. There was, however, no significant difference between the three varieties in the rate of *M. bolleyi* recovery.

TABLE 7.1: Frequency (% root segments infected) of *Microdochium bolleyi* re-isolated from the roots of 25 day old wheat plants. (Values are the mean of two replicates for each variety).

Variety	Inoculated	Control
Aroona	16.3	0
Miling	26.1	0
Kite	38.6	0
LSD (0.05)	57.2	-

7.3.2 Experiment 2

Symptoms on the Roots

Symptoms on the roots (Table 7.2) ranged from light brown cortical lesions (1.0 - 3.0 mm) to dark brown stelar lesions (3.0 - 5.0 mm). Roots also suffered general cortical and stelar discolouration, rather than distinct lesions. Some root tips were "spiked" with stelar browning and cortical degradation, and others were damaged without the "spiked" appearance. The most severe symptom, which occurred rarely, comprised cortical rotting and stelar browning about half way along the root, with constriction of the root over approximately 2.0 mm. This resulted in the root being almost severed at this point. The fungus also produced black chlamydospores within the cortical and epidermal cells of some roots. These spores occurred in groups over a small area of the root (<1.0 cm).

Condor was the most severely affected, displaying a wide range of symptoms (Table 7.2). (C8*MM)*MKRK)/18/15 and (C8*MM)*MKRK)/18/22 reacted similarly,

with moderate root damage. Miling, Oxley and (Fund*CP)*MKRK)/18/12 suffered only slight root damage.

TABLE 7.2: Incidence of symptoms on the roots of 35 day old wheat plants inoculated with *Microdochium bolleyi*. (Values are the total number of times each symptom was observed, over all replicates, ie. observations on fifteen plants for each variety and selection).

Symptom	Oxley	Miling	Condor	(Fund*CP)* MKRK)/8/ 12	(C8*MM)* MKRK)/18/ 15	(C8*MM)* MKRK)/18/ 22
Brown stele, cortex degraded, "spiked" root tip	0	0	3	1	0	0
Dark brown root tip, not "spiked"	1	0	1	0	0	1
Slight stelar browning	0	0	3	0	0	0
Light brown stelar lesions (3-5 mm)	0	0	3	0	0	0
Dark brown stelar lesions (3-5 mm)	1	0	1	0	3	3
Dark brown stelar lesions (>10 mm)	1	0	0	0	0	0
Light brown cortical lesions (1-3 mm)	0	4	5	1	2	2
Dark brown cortical lesions (1-4 mm)	0	0	3	1	1	4
Chlamydospores in cortical and epidermal cells	1	0	1	1	0	1
Root constricted, almost severed from plant	0	0	1	0	3	1

Re-isolation of Microdochium bolleyi

Roots of (C8*MM)*MKRK)/18/22 were most frequently infected with *M. bolleyi*, while Miling and Condor had the lowest incidence of infection (Table 7.3). However, there were no significant differences in infection levels between inoculated varieties or lines.

TABLE 7.3: Frequency (% root segments infected) of *Microdochium bolleyi* re-isolated from roots of 35 day old wheat plants. (Values are the mean of three inoculated or two uninoculated replicates for each variety and selection).

Variety or Selection	Inoculated	Control
Oxley	55.6	0
Miling	54.6	0
Condor	54.6	0
(Fund*CP)*MKRK)/8/12	66.7	0
(C8*MM)*MKRK)/18/15	67.8	0
(C8*MM)*MKRK)/18/22	71.0	0
LSD (0.05)	37.0	-

A second set of isolation plates was prepared, containing 1.0 cm root segments (from inoculated plants) displaying specific symptoms. Frequency of *M. bolleyi* infecting these roots was determined.

There were only two instances (out of 34 root segments plated) where *M. bolleyi* was not re-isolated from all such root samples. Brown root tips from (C8*MM)*MKRK)/18/22 were not infected with *M. bolleyi*, although this symptom on all other varieties or lines was associated with 100% recovery of *M. bolleyi* from the roots. On the same selection, only 50% of flaccid, decorticated roots with dark, brown stelar lesions (>5.0 mm in length) were infected with *M. bolleyi*, but roots of other varieties and selections with similar symptoms were all infected with *M. bolleyi*.

Condor displayed the most severe root damage, and all roots with specific

symptoms were infected with *M. bolleyi*. These symptoms included stelar and cortical lesions (2.0 - 3.0 mm), dark brown root tips and "spear" tips with associated stelar and cortical discolouration. *M. bolleyi* was isolated from all Condor roots containing black chlamydospores. Roots from this variety with lesions causing constriction and the near-severing of the root at the point of lesioning were all infected with *M. bolleyi*.

(Fund*CP)*MKRK)/18/12 roots with the following symptoms were all infected with *M. bolleyi*: "spear" tips, stelar and cortical lesions, chlamydospores within cortical and epidermal cells.

M. bolleyi was isolated from all the decorticated and lesioned (>5.0 mm) Oxley roots, along with those containing chlamydospores. Roots of (C8*MM)*MKRK)/18/15 displayed cortical lesions, stelar lesions and constricting lesions almost severing the root from the plant. All these roots were colonised by *M. bolleyi*.

Plant Dry Weight

(C8*MM)*MKRK)/18/15 was the only variety significantly influenced by infection with *M. bolleyi* (Table 7.4). Shoot weight of this selection was reduced significantly ($P<0.05$) by inoculation with *M. bolleyi*.

There were no significant differences between root weights of varieties, either inoculated or uninoculated (Table 7.4), but shoot weight of inoculated Oxley was significantly ($P<0.05$) less than that of all but Condor. However, growth of uninoculated Oxley was also significantly less than that of other varieties and selections, so the difference is unlikely to be due to infection by *M. bolleyi*. The shoots of inoculated Condor weighed significantly ($P<0.05$) less than those of (C8*MM)*MKRK)/18/15 and (C8*MM)*MKRK)/18/22.

7.3.3 Summary of Results

Inoculation of wheat plants with *M. bolleyi* showed that:

(1) *M. bolleyi* readily invaded root tissues, achieving high levels of infection. Thirty five days after inoculation, more than 55% of root segments tested were infected.

However, roots did not suffer extensive damage, and the effect of *M. bolleyi* on plant growth was negligible.

(2) *M. bolleyi* caused varying degrees of lesioning and root damage. Orange-brown lesions and discolouration, along with the formation of black chlamydospores in cortical and epidermal cells, were characteristic of infection with *M. bolleyi.*

(3) In these experiments, there were no significant varietal differences in the level of infection or the effect of *M. bolleyi* on root growth of plants.

TABLE 7.4: Dry weight (mg/plant) of 35 day old wheat plants inoculated with *Microdochium bolleyi* and that of uninoculated control plants. (Values are the mean of three inoculated and two uninoculated replicates for each variety and selection).

	INOCULATED			CONTROL		
Variety or Selection	**Shoot**	**Root**	**Total**	**Shoot**	**Root**	**Total**
Oxley	68.5	74.5	143.0	68.4	70.9	139.3
Miling	95.1	55.5	150.6	97.9	67.8	165.7
Condor	80.7	72.3	153.0	87.3	61.7	149.0
(Fund*CP)*MKRK)/8/12	98.3	72.8	171.1	99.0	64.1	163.1
(C8*MM)*MKRK)/18/15	108.0	55.5	163.5	137.5	58.0	195.5
(C8*MM)*MKRK)/18/22	107.3	67.1	174.4	95.1	47.6	142.7
LSD (0.05) Varieties	20.9	20.6	38.5	10.5	33.4	35.0
LSD (0.05) Treatments	20.3	27.1	40.0			

7.4 DISCUSSION

When inoculated with *M. bolleyi* alone, wheat roots did not suffer extensive damage. However, the range of symptoms included:

general orange-brown cortical discolouration

small (<5.0 mm) light and dark brown stelar and cortical lesions

"spiked" root tips with cortical degradation and stelar browning

orange-brown stunted root tips

lesions about half way along the root, where the root was constricted and almost severed from the plant

formation of black chlamydospores in the cortical and epidermal cells.

Isolations from roots showing these symptoms indicated that *M. bolleyi* was responsible for the damage.

Few of these symptoms could be specifically associated with infection by *M. bolleyi*, as they are similar to the lesions and rotting produced by other root attacking fungi (*Pythium* spp. and *Rhizoctonia solani*, for example). In fact, Waller (1979) found that *M. bolleyi* could be frequently isolated from the types of lesions that are usually attributed to infection by *Pythium* spp. The orange-brown colouration of some of the lesions, however, seems to be characteristic of *M. bolleyi* infection. Murray and Gadd (1981) also observed orange-brown lesions on the coleoptiles of plants inoculated with *M. bolleyi*.

Formation of black chlamydospores in cortical and epidermal cells is symptomatic of *M. bolleyi* infection. These were also noted by Murray (1981) and Murray and Gadd (1981). As chlamydospores are resting structures, these workers suggest that they are associated with negligible damage to root tissues. *M. bolleyi* seems unable to continue growing when confronted with the host plant response to infection, and responds by developing these dormant spores.

Under the conditions of Experiment 1, *M. bolleyi* invaded root tissues slowly, so it was not recovered within the first fourteen days. However, by 25 days, the fungus was readily isolated from a large proportion of the roots. The rate of root colonisation would, of course, depend on the experimental conditions. Fitt and Hornby (1978) found that *M. bolleyi* had invaded roots a week after plants were inoculated. In the field, *M. bolleyi* is probably one of the first fungi to infect seedling roots.

Kite sustained the most severe root damage in the first experiment, and root samples of this variety yielded the most *M. bolleyi*. In Experiment 2, Condor suffered the most severe root damage. However, there were no significant differences in *M. bolleyi*

infection between any of the varieties or lines.

The effect of *M. bolleyi* on plant growth was negligible. The selection (C8*MM)*MKRK)/18/15 was the only variety or line with growth significantly reduced by inoculation with *M. bolleyi*, although the fungus was readily recovered from the roots of all inoculated plants. The roots of (C8*MM)*MKRK)/18/15 sustained only minor damage, while *M. bolleyi* damaged the roots of Condor more severely, although the growth of Condor roots was not retarded.

It is doubtful that *M. bolleyi* is solely responsible for the low yields of these varieties and selections at field sites, or for shrivelling the grain of Miling. Furthermore, inhibition of lateral root growth, as observed in the field (Chapter 6), was not reproduced by inoculating wheat with *M. bolleyi*. Nevertheless, *M. bolleyi* achieves high levels of infection, and is frequently detected in field samples. It is possible that this fungus acts in conjunction with other organisms to cause the root damage observed under field conditions. The hypothesis that *M. bolleyi* augments root disease caused by *Bipolaris sorokiniana* was therefore tested under controlled conditions and in the field.

CHAPTER 8

INTERACTION BETWEEN *MICRODOCHIUM BOLLEYI* AND *BIPOLARIS SOROKINIANA*

8.1 POT EXPERIMENTS

8.1.1 INTRODUCTION

Microdochium bolleyi alone had negligible impact on plant growth, and the severity of root damage observed under field conditions was not reproduced by inoculation with this species (Chapter 7). However, *M. bolleyi* was one of the fungi most frequently isolated from field samples (Chapter 4), and may play some part in inhibiting lateral root growth under field conditions (Chapter 6). Experiments were therefore conducted to determine whether *M. bolleyi* augments root rot caused by *Bipolaris sorokiniana*, thus causing root damage similar to that observed in the field.

When plants are inoculated with two species of root rotting fungi, the following results are possible. The mixture may result in the same disease index as that achieved when plants are exposed to the "stronger" pathogen alone, or the severity of disease may be higher (ie. a synergistic interaction) or lower (ie. an antagonistic effect) than that brought about by the most damaging organism. Alternatively, disease may be produced by the additive effects of the two pathogens.

Three inoculation experiments were conducted in pots to determine whether there was a significant interaction between *M. bolleyi* and *B. sorokiniana* infecting wheat plants. A preliminary experiment (Experiment 1) involving a single wheat variety (Condor) was undertaken. Condor was inoculated with *M. bolleyi*, *B. sorokiniana*, both fungi, or neither. The second experiment (Experiment 2) involved a comparison between a variety moderately resistant to *B. sorokiniana* (Kite) and a moderately susceptible variety (Condor), to determine the influence of varietal reaction on the interaction between fungi. Experiment 3 had the same aims as Experiment 2, but inoculum was placed above

and below the seed, rather than below only. *B. sorokiniana* preferentially invades the subcrown internode, and this experiment tested the hypothesis that the interaction between fungi could be enhanced when stem tissues as well as roots were exposed to infection.

8.1.2 METHODS

Pre-germinated, surface-sterilised seed was planted in pots of steam-sterilised soil, as described in the General Methods for pot experiments. Each pot contained five seedlings. All pots were placed in the glasshouse in a waterbath maintained at 19±2°C.

Experiment 1 consisted of nine replicates of each treatment. Five replicates per treatment for each variety were used in Experiments 2 and 3.

Inoculation

Colonies of *M. bolleyi* and *B. sorokiniana* were grown on PDA plates for ten to sixteen days prior to preparation of inoculum. Spore suspensions were prepared for each fungus, as described in the General Methods, and for the combination of *M. bolleyi* and *B. sorokiniana*. Suspensions (or sterile 0.1% NaCl for the controls) were thoroughly mixed into soil and the soil incubated at 20°C for three to five days. Inoculated soil contained 306.8 *B. sorokiniana* spores/gram. The spore concentration of *M. bolleyi* was not determined, as this species produces enormous numbers of small spores in culture. Suspensions were heavily loaded with spores, and concentrations would have been greater than that determined for *B. sorokiniana.*

Experiments 1 & 2: 200 g of steam-sterilised soil was placed in each pot, and 200 g of the inoculum (or control) soil added. Five pre-germinated seeds were placed on top of the inoculum layer, and an additional 200 g of steam-sterilised soil added above the seed. This resulted in a seeding depth of approximately 3.0 cm, which promoted the development of long subcrown internodes.

Experiment 3: 200 g of steam-sterilised soil was added to each pot as before, followed by 200 g of the inoculum (or control) soil on which the seed was placed. Above

the seed, a further 200 g of inoculum soil was added, then another 50 g of steam-sterilised soil, resulting in a seeding depth of approximately 4.0 cm.

Sampling

Experiment 1: Three replicates of each treatment were sampled 25, 49 and 63 days after planting. Washed roots were scrutinised and symptoms noted. Twelve root segments (1.0 cm long) per replicate were removed (at random) for plating on RA medium. Subcrown internodes were rated for infection with *B. sorokiniana*, according to the technique described in the General Methods. All subcrown internodes were plated on isolation medium. Shoots and roots were dried and weighed.

Experiments 2 & 3: All pots were washed free of soil 28 days after planting. Subcrown internodes were rated for *B. sorokiniana* infection, and all were plated on RA medium. Twelve 1.0 cm long root samples were selected at random from each replicate, and placed on isolation medium. The roots and shoots were dried and weighed.

8.1.3 RESULTS

8.1.3.1 Experiment 1

Symptoms

25 days - Symptoms are described for seminal roots only, as no crown roots had formed, and subcrown internodes were still obscured by the coleoptile (Table 8.1).

Plants inoculated with *M. bolleyi* - Root mass and lateral root development was inferior to that of uninoculated plants, but appeared greater than that of plants inoculated with *B. sorokiniana* or *M. bolleyi* + *B. sorokiniana*. *M. bolleyi* did not interfere with seedling establishment.

Plants inoculated with *B. sorokiniana* - Plants were stunted, compared to those inoculated with *M. bolleyi* or the controls, and had a smaller root mass and less lateral root development than other treatments. *B. sorokiniana* reduced seedling establishment, with some seedlings dying prior to emergence.

Plants inoculated with *M. bolleyi* + *B. sorokiniana* - Root mass and plant height were comparable to that of control plants. Inoculation with both species interfered with seedling establishment, but to a lesser extent than did *B. sorokiniana* alone.

Controls - All plants were healthy with little or no root damage.

TABLE 8.1: Incidence of symptoms on roots and stem bases of 25 day old Condor wheat seedlings inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and on uninoculated control plants. (Values are the total number of times each symptom was observed over all replicates, ie. observations on fifteen plants for each treatment).

	INOCULATION TREATMENT			
Symptom	**Mb**	**Bs**	**MbBs**	**Control**
Brown root lesions (1.0 mm)	21	32	11	4
Brown root lesions (2.0-5.0 mm)	8	15	5	1
Brown root lesions (>1.0 cm)	0	0	1	0
Dark brown root tips	5	1	7	2
"Spear" root tips	4	1	2	1
Root cortex degraded	9	2	1	0
Flaccid roots	5	12	11	0
Lower leaf sheath lesions	0	4	16	0
Stem base lesions (1.0-3.0 mm)	0	45	26	0
Stem base lesions (4.0-7.0 mm)	0	20	5	0
Stem base lesions (8.0-13.0 mm)	0	5	0	0

49 days - Plants had produced crown roots by this stage, all of which were healthy except on plants inoculated with *B. sorokiniana* alone, where they were covered with small (<1.0 mm) brown lesions (Table 8.2). However, the incidence of symptoms at 49 days was actually lower than at 25 days (Table 8.1).

Plants inoculated with *M. bolleyi* - Root mass was comparable to that of controls and plants displayed little lesioning or general discolouration.

Plants inoculated with *B. sorokiniana* - Plants were stunted with roots, stem

bases and lower leaf sheaths lesioned. Many seminal roots displayed small, brown lesions.

Plants inoculated with *M. bolleyi* + *B. sorokiniana* - Little root damage was visible, apart from general discolouration over most root systems.

Controls - All plants were healthy.

TABLE 8.2: Incidence of symptoms on roots and stem bases of 49 day old Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and on uninoculated control plants. (Values are the total number of times each symptom was observed, over all replicates, ie. observations on fifteen plants for each treatment).

	INOCULATION TREATMENT			
Symptom	**Mb**	**Bs**	**MbBs**	**Control**
Brown seminal root lesions (1.0 mm)	0	4	5	0
Brown seminal root lesions (2.0-4.0 mm)	2	8	4	1
Brown seminal root lesions (>1.0 cm)	0	6	0	0
Dark brown root tips	0	3	0	0
"Spear" root tips	2	4	1	1
Flaccid roots	0	3	0	0
Number of undamaged plants	0	0	0	13
Number of unemerged plants	0	4	2	0
Plants with discoloured roots	15	14	12	0
Lower leaf sheath lesions	0	1	0	0
Stem base lesions (1.0-2.0 mm)	1	5	0	0
Stem base lesions (>1.0 cm)	0	1	0	0
Crown roots	clean	lesioned	clean	clean

63 days - By this time, plants displayed more severe root and subcrown internode damage than was evident at either 25 or 49 days. Incidence of symptoms was not tabulated for the 63 day old plants.

Plants inoculated with *M. bolleyi* - All plants were generally healthy with little root damage, and each had a large root mass compared to plants inoculated with *B.*

sorokiniana or *M. bolleyi* + *B. sorokiniana*. Lateral roots were well-developed.

Plants inoculated with *B. sorokiniana* - Many seminal roots were lesioned, with brown tips or flaccid sections (ie. root with gray, water-soaked appearance, without actual rotting or lesioning), and general discolouration. Most crown roots were healthy, but some did have a few small lesions. Subcrown internodes were severely lesioned.

Plants inoculated with *M. bolleyi* + *B. sorokiniana* - Little damage occurred, except general discolouration over most root systems. Subcrown internodes sustained only slight lesioning.

Controls - Plants were generally healthy with a large root mass and no lesioning or other damage. Some root systems displayed a little general discolouration.

Re-isolation of Microdochium bolleyi and Bipolaris sorokiniana

Root and subcrown internode samples of uninoculated plants were not infected by either species. Plants inoculated with *M. bolleyi* + *B. sorokiniana* were colonised significantly less by *B. sorokiniana* than were those inoculated with *B. sorokiniana* alone (Table 8.3). After 25 days, inoculation with *M. bolleyi* + *B. sorokiniana* reduced *B. sorokiniana* infection of seminal roots by 57%, which was significant ($P<0.05$). No crown roots had formed on 25 day old seedlings, nor were subcrown internodes evident.

By 49 days, seminal roots and subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 51% and 63% less *B. sorokiniana*, respectively, than plants inoculated with *B. sorokiniana* alone (Table 8.3), but only the latter was significant ($P<0.05$). The frequency of *M. bolleyi* on the seminal roots of plants was reduced 84% by inoculation with *M. bolleyi* + *B. sorokiniana*, and this difference was significant ($P<0.05$). Neither species was isolated from crown roots of 49 day old plants.

At 63 days, plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by significantly less *B. sorokiniana* than those inoculated with *B. sorokiniana* alone (Table 8.3): *B. sorokiniana* frequency was reduced by 53% on seminal roots ($P<0.05$), 60% on subcrown internodes ($P<0.05$) and 58% on the crown roots (NS). Similarly, *M. bolleyi* frequency was reduced at 63 days when plants were inoculated with *M. bolleyi* + *B.*

sorokiniana: seminal roots were infected by 38% less *M. bolleyi* (NS), subcrown internodes by 53% less ($P<0.05$) and crown roots by 29% less *M. bolleyi* (NS).

On isolation medium, both fungi grew from the roots of plants that had been inoculated with *M. bolleyi* + *B. sorokiniana* (Plate 8.1). Colonies of *M. bolleyi* were more frequent than those of *B. sorokiniana*, but both species grew from some of the individual root segments. There was no antagonism between the two species in culture. Hyphae grew together, and no zone of inhibition developed between colonies. This occurred whether the fungi were growing from infected host tissue on isolation plates (Plate 8.1), or were inoculated together onto PDA plates in the absence of host tissue.

Plant Dry Weight

At 25 days, plants inoculated with *B. sorokiniana* produced 37% less shoot and 32% less total dry matter than those inoculated with *M. bolleyi* + *B. sorokiniana*, but neither of these differences were significant (Table 8.4). The same trend was apparent at 49 days: plants inoculated with *B. sorokiniana* produced 40% less shoot ($P<0.05$), 54% less root ($P<0.05$) and 46% less total dry matter ($P<0.05$) than plants inoculated with both species.

By 63 days, plants inoculated with *M. bolleyi* + *B. sorokiniana* had produced 49% more shoot ($P<0.05$), 31% more root (NS) and 42% more total dry matter ($P<0.05$) than those inoculated with *B. sorokiniana* alone (Table 8.4). In some cases, plants inoculated with *M. bolleyi* alone weighed less than those inoculated with *M. bolleyi* + *B. sorokiniana*, but these differences were only significant ($P<0.05$) at 49 days, when plants inoculated with *M. bolleyi* weighed 42% less than those inoculated with *M. bolleyi* + *B. sorokiniana.*

Weights of plants inoculated with *M. bolleyi* + *B. sorokiniana* were never significantly less than those of the control plants, whereas weights of plants inoculated with either species alone, especially *B. sorokiniana*, were generally significantly less than those of the controls (Table 8.4). *B. sorokiniana* had a greater impact on plant growth than did *M. bolleyi*. At 25 days, plants inoculated with *B. sorokiniana* weighed 40% less ($P<0.05$) than the controls, whereas those inoculated with *M. bolleyi* weighed only 31%

less (NS) than the uninoculated control plants. Both fungi had a similar effect on plants at 49 days: *B. sorokiniana* reduced plant growth by 46% ($P<0.05$) and *M. bolleyi* by 43% ($P<0.05$). By 63 days, *M. bolleyi* had no significant effect on plant weight, while *B. sorokiniana* reduced total plant weight by 49% ($P<0.05$).

TABLE 8.3: Frequency (% plant segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) re-isolated from seminal roots (SRT), crown roots (CRT) and subcrown internodes (SCI) of 25, 49 and 63 day old Condor wheat plants inoculated with *M. bolleyi* (Mb), *B. sorokiniana* (Bs) or both (MbBs), and from uninoculated control plants. (Values are the mean of three replicates for each treatment). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations.

		INOCULATION TREATMENT								
		Mb		Bs		MbBs		Control		LSD (0.05)
25 DAYS										
SRT	**Mb**	46.0	(42.6)	0	(10.0)	40.4	(38.9)	0	(10.0)	15.8
	Bs	0	(10.0)	74.8	(64.7)	32.5	(34.6)	0	(10.0)	16.3
49 DAYS										
SRT	**Mb**	35.6	(36.1)	0	(10.0)	5.6	(14.7)	0	(10.0)	11.9
	Bs	0	(10.0)	50.0	(45.0)	24.4	(28.2)	0	(10.0)	19.0
CRT	**Mb**	0	(10.0)	0	(10.0)	0	(10.0)	0	(10.0)	-
	Bs	0	(10.0)	0	(10.0)	0	(10.0)	0	(10.0)	-
SCI	**Mb**	6.7	(13.2)	0	(6.6)	9.5	(15.1)	0	(6.6)	20.3
	Bs	0	(6.6)	55.6	(48.7)	20.6	(26.9)	0	(6.6)	14.8
63 DAYS										
SRT	**Mb**	72.2	(59.9)	0	(10.0)	44.5	(41.3)	0	(10.0)	22.0
	Bs	0	(10.0)	83.3	(66.9)	38.9	(38.0)	0	(10.0)	21.8
CRT	**Mb**	94.4	(75.3)	0	(10.0)	66.7	(56.7)	0	(10.0)	23.7
	Bs	0	(10.0)	66.7	(56.7)	27.8	(29.6)	0	(10.0)	37.2
SCI	**Mb**	88.9	(74.1)	0	(6.6)	41.7	(37.2)	0	(6.6)	33.4
	Bs	0	(6.6)	100.0	(83.7)	40.0	(38.9)	0	(6.6)	10.6

TABLE 8.4: Dry weight (mg/plant) of 25, 49 and 63 day old Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and of uninoculated control plants. (Values are the mean of three replicates for each treatment).

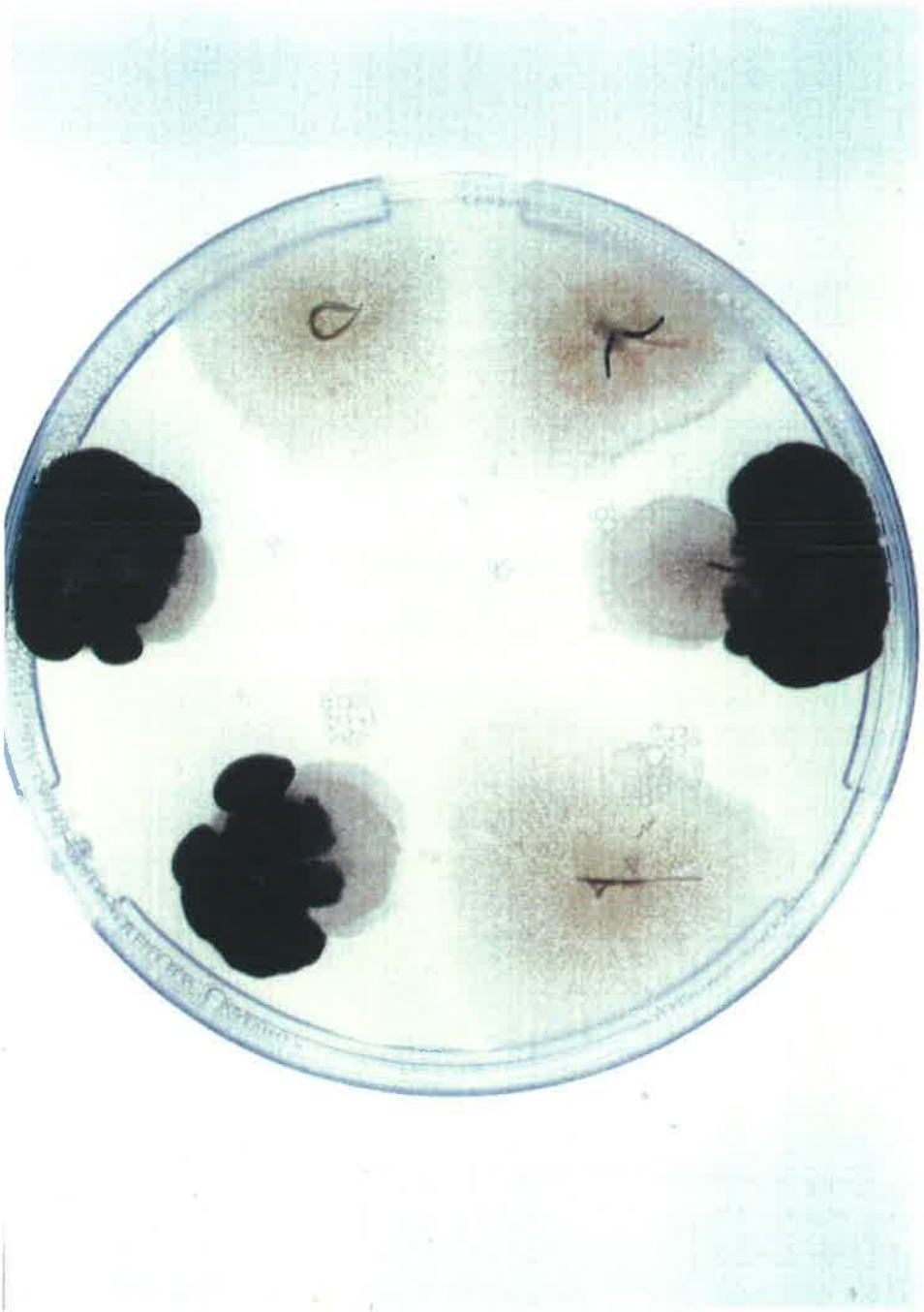
	INOCULATION TREATMENT				
	Mb	**Bs**	**MbBs**	**Control**	**LSD (0.05)**
25 DAYS					
Shoot	45.3	38.3	60.5	65.1	22.5
Root	9.8	10.2	10.7	15.3	7.0
Total	55.1	48.5	71.2	80.4	28.6
49 DAYS					
Shoot	94.1	103.0	170.9	171.9	54.0
Root	53.1	38.3	84.2	88.2	30.3
Total	147.2	141.3	255.1	260.1	76.0
63 DAYS					
Shoot	173.8	103.6	204.6	223.4	75.3
Root	185.7	89.3	130.1	154.9	73.5
Total	359.5	192.9	334.7	378.3	122.5

TABLE 8.5: Disease rating (%) of 25, 49 and 63 day old Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and that of uninoculated control plants. (Values are the mean of three replicates for each treatment). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations.

	INOCULATION TREATMENT				
	Mb	**Bs**	**MbBs**	**Control**	**LSD (0.05)**
25 days		no subcrown internodes visible			
49 days	0 (12.9)	22.8 (30.6)	11.8 (19.8)	0 (12.9)	14.3
63 days	0 (12.9)	85.4 (67.6)	17.5 (24.7)	0 (12.9)	15.9

PLATE 8.1: *Microdochium bolleyi* (light orange colonies) and *Bipolaris sorokiniana* (black colonies) growing from roots of plants that had been inoculated with both species of fungus. *M. bolleyi* was isolated more frequently than was *B. sorokiniana*, and both species grew from some of the roots. No antagonism occurred between hyphae of the two fungi on agar plates.

Plate 8.1



Disease Rating

Values for disease rating could not be calculated at 25 days, as subcrown internodes were not visible. At 49 days, disease rating was reduced by 48% on plants inoculated with both fungi compared to those inoculated with *B. sorokiniana* alone, but this was not statistically significant (Table 8.5). However, by 63 days, the disease rating was reduced by 80%, which was significant ($P<0.05$). Neither control or *M. bolleyi* inoculated plants suffered subcrown internode lesioning.

8.1.3.2 Experiment 2

Re-isolation of Microdochium bolleyi and Bipolaris sorokiniana

Kite - Seminal roots and subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 48% and 73% less *B. sorokiniana*, respectively, than those inoculated with *B. sorokiniana* alone (Table 8.6). Both these differences were statistically significant ($P<0.05$). There was no significant difference in the level of *M. bolleyi* infection of seminal roots between plants inoculated with *M. bolleyi* and those inoculated with *M. bolleyi* + *B. sorokiniana*. However, subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 53% less *M. bolleyi* ($P<0.05$) than those inoculated with *M. bolleyi* alone.

Condor - Seminal roots of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 51% less *M. bolleyi* and 54% less *B. sorokiniana* compared to plants inoculated with either species alone (Table 8.6), but only the latter difference was significant ($P<0.05$). Subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 58% less ($P<0.05$) *M. bolleyi*. There was no significant difference in *B. sorokiniana* frequency on subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* or those inoculated with *B. sorokiniana* alone.

Plant Dry Weight

Kite - There were no significant differences between treatments, but plants inoculated with *M. bolleyi* + *B. sorokiniana* weighed marginally more than those

inoculated with *B. sorokiniana* alone (Table 8.7).

Condor - Plants inoculated with *M. bolleyi* + *B. sorokiniana* weighed significantly less ($P<0.05$) than those inoculated with *B. sorokiniana* alone, and those inoculated with *M. bolleyi* weighed more than those inoculated with both species of fungi, but not significantly (Table 8.7). Weight of inoculated plants was not significantly different from that of the controls.

TABLE 8.6: Frequency (% plant segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) re-isolated from seminal roots (SRT) and subcrown internodes (SCI) of 25 day old Kite and Condor wheat plants inoculated with *M. bolleyi* (Mb), *B. sorokiniana* (Bs) or both (MbBs), and from uninoculated control plants. (Values are the mean of five replicates for each treatment). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations.

		INOCULATION TREATMENT								
		Mb		Bs		MbBs		Control		LSD (0.05)
KITE										
SRT	Mb	33.3	(34.6)	0	(10.0)	41.7	(40.1)	0	(10.0)	8.6
	Bs	0	(10.0)	39.7	(38.0)	20.0	(25.4)	0	(10.0)	10.8
SCI	Mb	72.7	(62.2)	0	(6.6)	34.0	(33.6)	0	(6.6)	17.6
	Bs	0	(6.6)	85.0	(69.5)	23.0	(25.5)	0	(6.6)	13.8
CONDOR										
SRT	Mb	23.6	(27.4)	0	(10.0)	11.7	(19.5)	0	(10.0)	11.5
	Bs	0	(10.0)	28.9	(31.8)	13.3	(20.6)	0	(10.0)	10.6
SCI	Mb	55.0	(49.7)	0	(6.6)	23.3	(25.7)	0	(6.6)	18.7
	Bs	0	(6.6)	53.3	(48.8)	71.7	(61.4)	0	(6.6)	21.7

Disease Rating

Kite - The disease rating of plants inoculated with both fungi was 72% lower than that of plants inoculated with *B. sorokiniana* alone (Table 8.8). This difference was significant ($P<0.05$).

Condor - Plants inoculated with *M. bolleyi* + *B. sorokiniana* had a disease rating 9% lower than that of plants inoculated with *B. sorokiniana* alone, but this difference was not significant (Table 8.8).

TABLE 8.7: Dry weight (mg/plant) of 28 day old Kite and Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and of uninoculated control plants. (Values are the mean of five replicates for each treatment).

	INOCULATION TREATMENT				
	Mb	**Bs**	**MbBs**	**Control**	**LSD (0.05)**
KITE					
Shoot	721.6	689.4	690.2	739.1	70.9
Root	155.6	167.1	172.1	166.8	49.0
Total	877.2	856.5	862.3	905.9	107.1
CONDOR					
Shoot	764.7	745.7	676.0	775.4	133.3
Root	445.2	487.1	262.5	331.2	203.0
Total	1209.9	1232.8	938.5	1106.6	286.9

TABLE 8.8: Disease rating (%) of 28 day old Kite and Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and that of uninoculated control plants. (Values are the mean of five replicates for each treatment). Values in brackets are $\sqrt{(x+0.5)}$ transformed means, and the LSD (0.05) is based on these transformations.

	INOCULATION TREATMENT				
	Mb	**Bs**	**MbBs**	**Control**	**LSD (0.05)**
Kite	0 (0.7)	25.0 (5.0)	7.0 (2.6)	0 (0.7)	1.0
Condor	0 (0.7)	14.6 (3.8)	13.3 (3.3)	0 (0.7)	1.7

8.1.3.3 Experiment 3

Re-isolation of Microdochium bolleyi and Bipolaris sorokiniana

Kite - Seminal roots and subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 29% and 55% less *B. sorokiniana*, respectively, than those inoculated with *B. sorokiniana* alone (Table 8.9). These differences were both significant ($P<0.05$). There was no significant difference in the frequency of *M. bolleyi* on subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* or with *M. bolleyi* alone.

Condor - Seminal roots and subcrown internodes of plants inoculated with *M. bolleyi* + *B. sorokiniana* were infected by 56% and 70% less *B. sorokiniana*, respectively, than plants inoculated with *B. sorokiniana* alone (Table 8.9), and these differences were both significant ($P<0.05$). *M. bolleyi* infection of seminal roots and subcrown internodes was reduced 33% ($P<0.05$) and 17% (NS), respectively, by inoculation with both species.

Plant Dry Weight

Kite - Plants inoculated with *M. bolleyi* + *B. sorokiniana* weighed 17% more than those inoculated with *B. sorokiniana* alone, but this was not statistically significant (Table 8.10). Inoculation with *M. bolleyi* resulted in a total plant weight 21% greater ($P<0.05$) than that of plants inoculated with both fungi. Weight of inoculated plants was not significantly less than that of the controls, and inoculation with *M. bolleyi* actually led to a significant ($P<0.05$) increase in the total weight of Kite. *B. sorokiniana* did, however, reduce total plant weight by 15% (NS).

Condor - Inoculation with *M. bolleyi* + *B. sorokiniana* resulted in a similar plant weight to that of plants inoculated with *B. sorokiniana* (Table 8.10). Plants inoculated with *M. bolleyi* weighed more than those inoculated with *M. bolleyi* + *B. sorokiniana*, but this was not significant. Inoculation did not significantly affect plant growth, and inoculation with *B. sorokiniana* reduced plant weight by only 2%.

TABLE 8.9: Frequency (% plant segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) re-isolated from seminal roots (SRT) and subcrown internodes (SCI) of 28 day old Kite and Condor wheat plants inoculated with *M. bolleyi* (Mb), *B. sorokiniana* (Bs) or both (MbBs), and from uninoculated control plants. (Values are the mean of five replicates for each treatment). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations.

		INOCULATION TREATMENT								
		Mb		Bs		MbBs		Control		LSD (0.05)
KITE										
SRT	**Mb**	23.9	(27.9)	0	(10.0)	40.0	(39.1)	0	(10.0)	9.7
	Bs	0	(10.0)	90.0	(71.7)	64.0	(53.5)	0	(10.0)	6.9
SCI	**Mb**	43.3	(39.3)	0	(6.6)	41.7	(40.1)	0	(6.6)	34.0
	Bs	0	(6.6)	48.3	(44.0)	21.7	(24.7)	0	(6.6)	14.4
CONDOR										
SRT	**Mb**	78.1	(62.9)	0	(10.0)	52.1	(46.3)	0	(10.0)	14.1
	Bs	0	(10.0)	92.7	(75.5)	40.6	(38.5)	0	(10.0)	13.6
SCI	**Mb**	50.0	(45.1)	0	(6.6)	41.3	(39.8)	0	(6.6)	28.1
	Bs	0	(6.6)	78.3	(65.4)	23.7	(25.8)	0	(6.6)	17.8

Disease Rating

Kite - There was no significant difference in the disease rating of plants inoculated with *B. sorokiniana* or with *M. bolleyi* + *B. sorokiniana*, and inoculation with *M. bolleyi* resulted in a disease rating of 0% (Table 8.11).

Condor - Inoculation with *M. bolleyi* + *B. sorokiniana* resulted in a disease rating 32% lower than that of plants inoculated with *B. sorokiniana* alone, but this was not significant (Table 8.11). Disease rating of plants inoculated with *M. bolleyi* alone was 0%.

M. bolleyi alone caused no visible damage to subcrown internodes, whereas *B. sorokiniana* by itself and in combination with *M. bolleyi* produced significant disease.

TABLE 8.10: Dry weight (mg/plant) of 28 day old Kite and Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and of uninoculated control plants. (Values are the mean of five replicates for each treatment).

	INOCULATION TREATMENT				
	Mb	**Bs**	**MbBs**	**Control**	**LSD (0.05)**
KITE					
Shoot	113.5	77.2	91.3	84.4	22.6
Root	69.3	42.9	53.1	57.0	16.1
Total	182.8	120.1	144.4	141.4	37.0
CONDOR					
Shoot	109.6	106.4	100.2	113.3	26.5
Root	81.1	68.9	61.5	65.6	24.2
Total	190.7	175.3	161.7	179.0	47.1

TABLE 8.11: Disease rating (%) of 28 day old Kite and Condor wheat plants inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs), and that of uninoculated control plants. (Values are the mean of five replicates for each treatment). Values in brackets are $\sqrt{(x+0.5)}$ transformed means, and the LSD (0.05) is based on these transformations.

	INOCULATION TREATMENT				
	Mb	**Bs**	**MbBs**	**Control**	**LSD (0.05)**
Kite	0 (0.7)	8.8 (2.5)	9.6 (2.7)	0 (0.7)	1.9
Condor	0 (0.7)	10.0 (3.0)	6.8 (2.3)	0 (0.7)	1.2

8.1.3.4 Summary of Results

Inoculating plants with *M. bolleyi*, *B. sorokiniana*, both or neither showed that:

(1) Inoculation with *M. bolleyi* + *B. sorokiniana* usually resulted in less root and subcrown internode damage than that brought about by inoculation with *B. sorokiniana* alone.

(2) Plants inoculated with *M. bolleyi* + *B. sorokiniana* were colonised significantly less by *B. sorokiniana* than those inoculated with *B. sorokiniana* alone.

(3) Dry shoot and root weights of plants inoculated with both species of fungus were greater than those of plants inoculated with *B. sorokiniana* alone.

(4) Disease rating, measured as the extent of subcrown internode lesioning caused by *B. sorokiniana*, was reduced in the presence of *M. bolleyi*.

(5) To some extent, inoculation with both species also reduced the frequency of *M. bolleyi* infection.

(6) Inoculation with *M. bolleyi* resulted in a disease rating of 0%, indicating that this species does not visibly damage subcrown internode tissue, at least under the conditions of these experiments.

(7) Kite is moderately resistant to *B. sorokiniana* and Condor moderately susceptible, but there was an inconsistent relationship between both level of *B. sorokiniana* infection and disease rating with the reported varietal reactions to *B. sorokiniana*.

(8) Varietal reaction to *B. sorokiniana* had inconsistent effects on the interaction between *M. bolleyi* and *B. sorokiniana*.

8.2 PETRI PLATE EXPERIMENT

8.2.1 INTRODUCTION

Wheat seedlings were grown under controlled conditions and inoculated with *Microdochium bolleyi*, *Bipolaris sorokiniana*, both fungi or neither species in Petri plates. Two varieties of wheat, Kite and Machete, were used in this experiment, the former moderately resistant to *B. sorokiniana* and the latter susceptible (J. Lewis and A. J. Rathjen, personal communication; Dubé and Brooks, 1986; Wallwork, 1989; P. J. L. Whittle, personal communication). The influence of varietal reaction to *B. sorokiniana* on the interaction between *M. bolleyi* and *B. sorokiniana* could thus be examined, and the effect of the fungi on seedlings followed more closely.

8.2.2 METHODS

Inoculation and Growing Conditions

The experiment consisted of five replicate plates of each variety for the four inoculation treatments, including the uninoculated control plates. The general techniques were modified from Christensen *et al.* (1988).

Seed was surface-sterilised (Speakman and Kruger, 1983) and germinated at 20°C on Petri plates (9.0 cm in diameter) containing 2% TWA. Five seeds were placed across the centre of each plate while the agar was still liquid, allowing seeds to become partially embedded in the agar. Plates were incubated in stacks of ten, bound together with rubber bands, and placed on edge so that the agar surface was vertical and roots could then grow down, across the agar. Plates were incubated in this manner for five days, by which time the roots were 3.0 - 4.0 cm long.

Those plates containing five healthy seedlings free of visible contaminants were inoculated with *M. bolleyi* alone, *B. sorokiniana* alone, both species or neither. Spores from *B. sorokiniana* isolate #3061 and *M. bolleyi* #3071 (as described in the General Methods) were smeared across the TWA surface in a band approximately 0.5 cm wide, in the root zone, 3.0 cm below the seed. Inoculated and uninoculated plates were returned to the 20°C incubator, placed on edge as before, for a further three days. After this time, they were placed on the laboratory bench at room temperature to receive diffuse sunlight, preventing chlorosis of tissues.

Sampling and Measurements

Eighteen days after inoculation, seedlings were carefully removed from the agar. The number of leaves on each seedling was counted, and measurements made using a Mitutoyo® digimatic caliper. The length of the longest seminal root axis was measured, as was the maximum shoot length and the length of the coleoptile that had sustained lesioning. Whole plants from each replicate plate were dried and weighed.

8.2.3 RESULTS

B. sorokiniana grew and sporulated extensively on the agar and on the seedlings of plates inoculated with this species alone, but grew poorly on plates and seedlings inoculated with *M. bolleyi* + *B. sorokiniana* (Plate 8.2). This was true of both Kite and Machete seedlings.

Seedlings of Kite and Machete inoculated with *M. bolleyi* or *B. sorokiniana* alone produced significantly ($P<0.05$) fewer leaves than the controls, while *M. bolleyi* + *B. sorokiniana* did not affect leaf number (Table 8.12). Kite seedlings inoculated with *B. sorokiniana* alone had significantly ($P<0.05$) shorter roots than the controls, while *M. bolleyi* or *M. bolleyi* + *B. sorokiniana* had no effect on the root length. Inoculation had no significant effect on the root length of Machete seedlings. Shoot length of Machete seedlings was significantly ($P<0.05$) reduced by all inoculation treatments, more so by *B. sorokiniana* than by either *M. bolleyi* or *M. bolleyi* + *B. sorokiniana*. No treatment significantly affected shoot length of Kite. Seedling weights did not differ between treatments for either variety (Table 8.12).

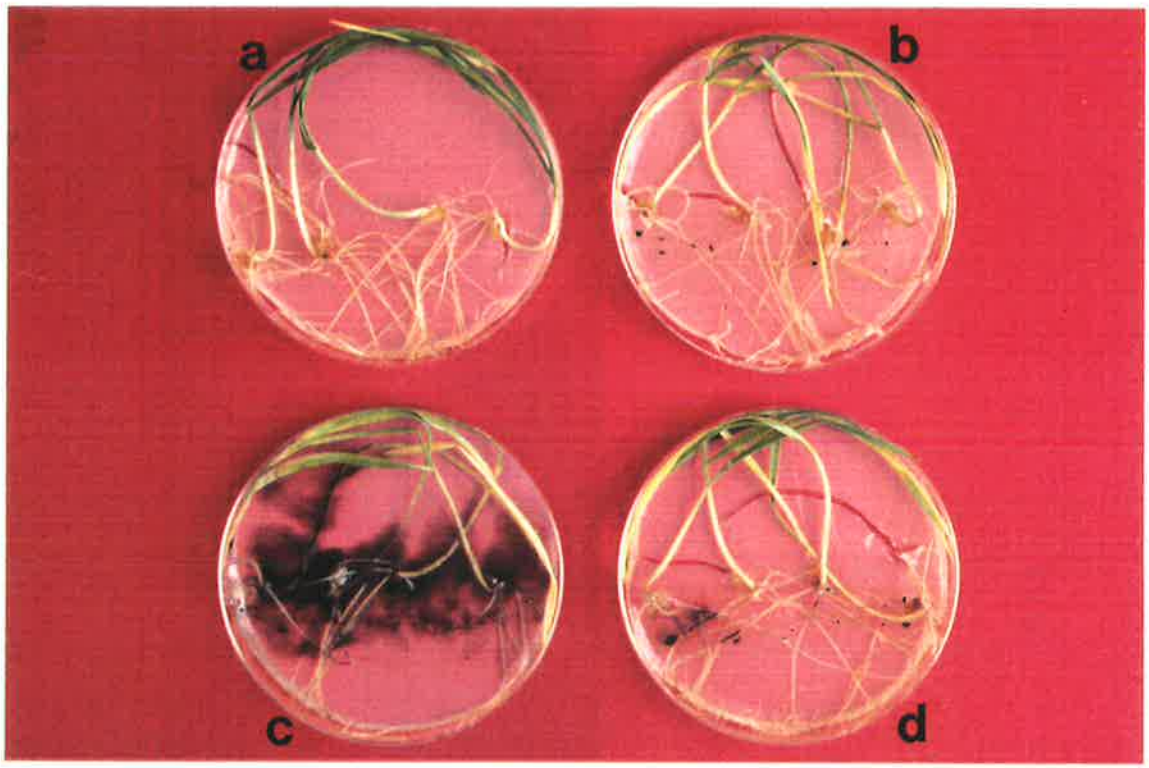
The major difference between treatments was in the percentage of the coleoptile lesioned. *M. bolleyi* alone caused negligible lesioning of coleoptiles (Table 8.12). *B. sorokiniana* produced significantly ($P<0.05$) more lesioning on both varieties than did *M. bolleyi*. Machete seedlings inoculated with *B. sorokiniana* sustained 40% more coleoptile lesioning than did those inoculated with *M. bolleyi* + *B. sorokiniana*. This was statistically significant ($P<0.05$). Coleoptiles of Kite inoculated with *M. bolleyi* + *B. sorokiniana* sustained 73% less ($P<0.05$) lesioning than those of plants inoculated with *B. sorokiniana* alone.

Although seedlings inoculated with *M. bolleyi* + *B. sorokiniana* had better root growth than those inoculated with *B. sorokiniana* alone (Plate 8.3), these differences were rarely significant. The presence of *M. bolleyi* did, however, significantly reduce the extent of coleoptile damage caused by *B. sorokiniana*, and alleviated the amount of leaf damage incurred (Plate 8.3).

PLATE 8.2: Machete (**A**) and Kite (**B**) seedlings were grown on Petri plates containing tapwater agar. These seedlings were uninoculated (**a**), inoculated with *Microdochium bolleyi* (**b**), *Bipolaris sorokiniana* (**c**) or *M. bolleyi* + *B. sorokiniana* (**d**). *B. sorokiniana* grew extensively on plates inoculated with this species alone (**c**), but grew poorly on plates that were also inoculated with *M. bolleyi* (**d**).

Plate 8.2

A



B

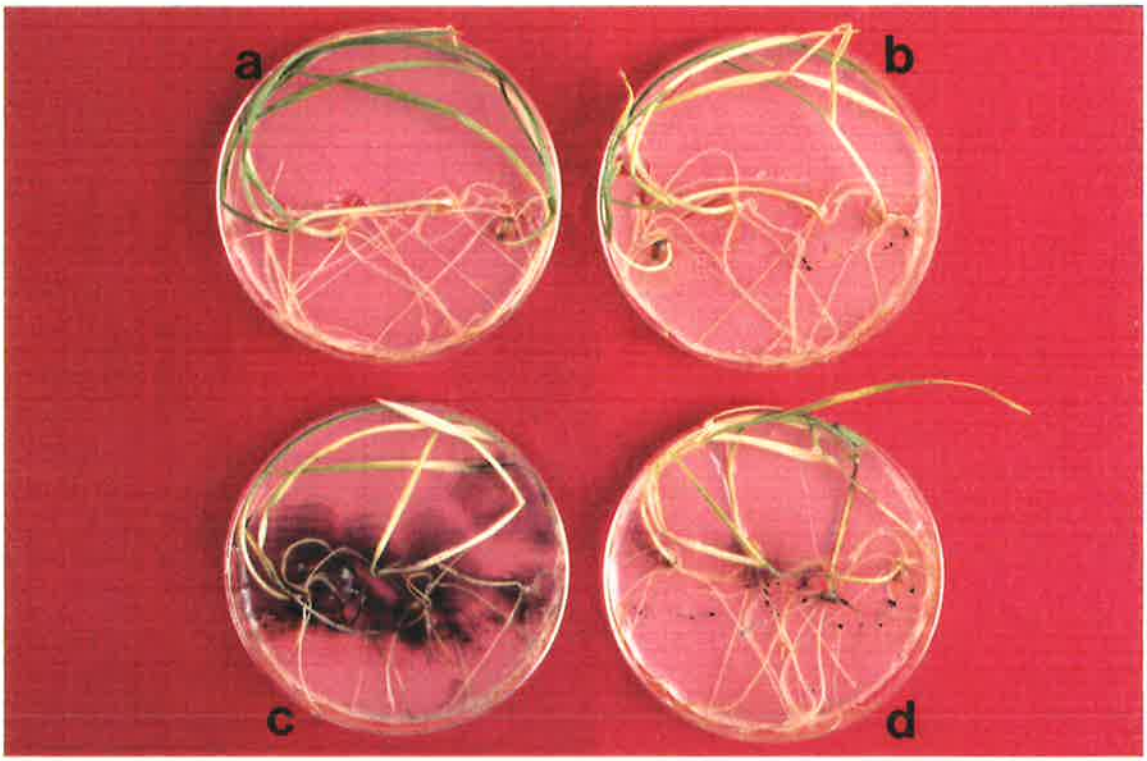
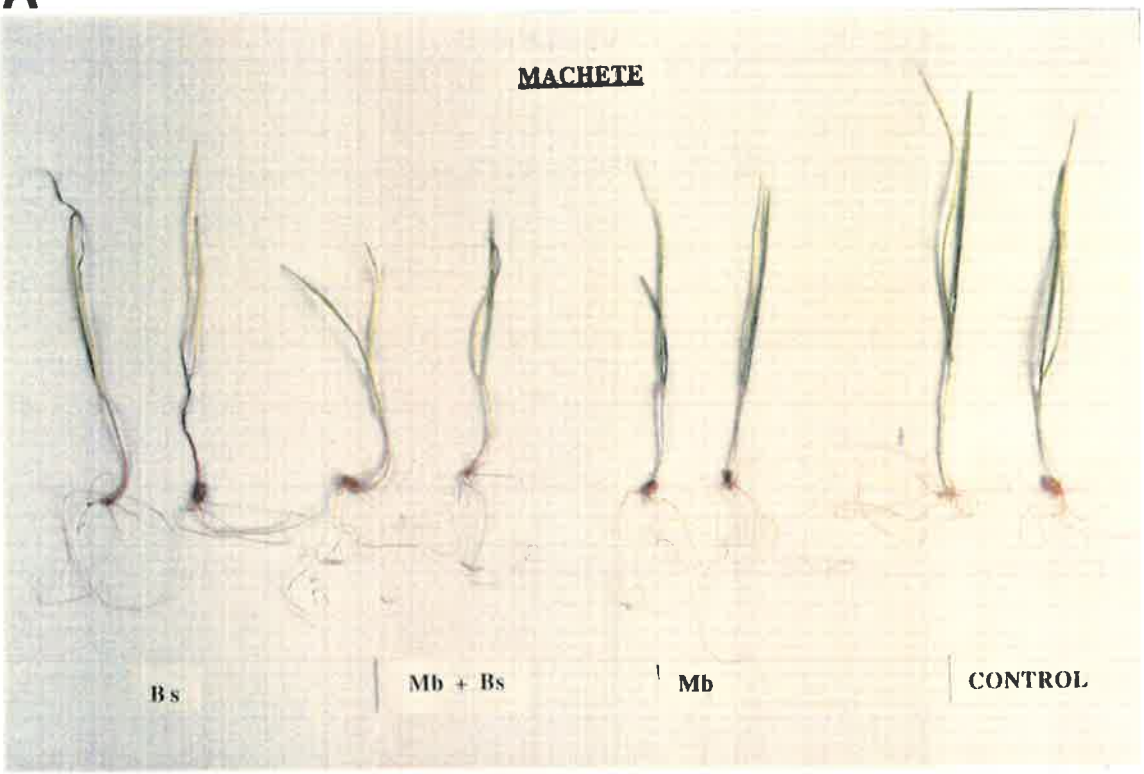


PLATE 8.3: Machete (**A**) and Kite (**B**) seedlings from Petri plates (Plate 8.2) inoculated with *Microdochium bolleyi* (**Mb**), *Bipolaris sorokiniana* (**Bs**), both (**Mb + Bs**) or neither (**Control**) fungus. Root growth of seedlings inoculated with *M. bolleyi* + *B. sorokiniana* was greater than that of plants inoculated with *B. sorokiniana* alone. *B. sorokiniana* caused extensive damage to the coleoptile, whereas *M. bolleyi* or *M. bolleyi* + *B. sorokiniana* did not. Seedlings inoculated with *B. sorokiniana* alone were chlorotic, with shrivelled leaves, but those inoculated with *M. bolleyi* or *M. bolleyi* + *B. sorokiniana* did not suffer the same degree of leaf damage.

Plate 8.3

A



B

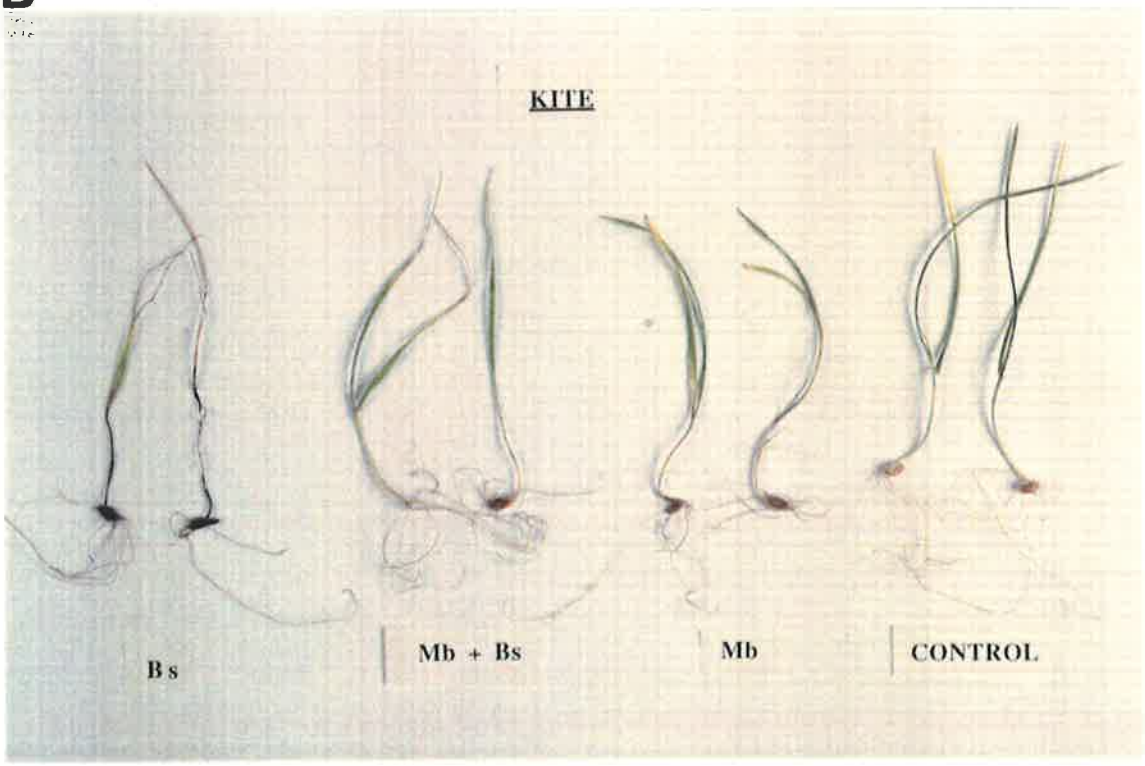


TABLE 8.12: Number of leaves/plant, maximum root and shoot length (mm), % coleoptile lesioned and plant dry weight (mg/plant) of Kite and Machete seedlings inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs) or both (MbBs) in Petri plates, and that of uninoculated control seedlings, eighteen days after inoculation. (Values are the mean of five replicates for each treatment).

Treatment	Leaves /Plant	Root Length (mm)	Shoot Length (mm)	% Coleoptile Lesioned	Dry Weight (mg/plant)
KITE					
Mb	1.7	81.0	105.0	11.6	15.6
Bs	1.5	68.0	98.6	73.1	17.1
MbBs	1.9	85.4	103.5	19.8	15.0
Control	2.2	96.8	136.6	0	20.2
LSD (0.05)	0.4	26.3	18.0	23.8	9.6
MACHETE					
Mb	1.8	85.5	109.2	4.6	15.5
Bs	1.7	97.9	105.6	55.0	18.4
MbBs	1.9	92.1	119.7	33.0	14.5
Control	2.1	96.0	126.3	0.1	20.5
LSD (0.05)	0.3	23.9	22.6	16.1	7.1

8.2.3.1 Summary of Results

Infection of wheat seedlings in Petri plates with *M. bolleyi* and *B. sorokiniana* indicated that:

(1) Inoculation with *B. sorokiniana* resulted in seedlings with fewer leaves, and shorter roots and shoots than those inoculated with *M. bolleyi* + *B. sorokiniana.*

(2) Seedling dry weight was not significantly affected by any inoculation treatment.

(3) Inoculation with *M. bolleyi* significantly reduced the extent of coleoptile lesioning caused by *B. sorokiniana.*

(4) *M. bolleyi* alone caused negligible damage to the coleoptiles.

(5) The effect of *B. sorokiniana* on plant growth was not consistent with the known varietal reactions to this fungus.

8.3 FIELD EXPERIMENTS

8.3.1 INTRODUCTION

Experiments under controlled conditions in the glasshouse and laboratory indicated that plants inoculated with *Microdochium bolleyi* + *Bipolaris sorokiniana* suffered less damage than those inoculated with *B. sorokiniana* alone. In 1988 and 1989, trials were sown at two sites in the Murray Mallee (Figure 3.1) to test this hypothesis under field conditions.

Plots were sown on fumigated and untreated areas, and inoculated with *M. bolleyi*, *B. sorokiniana*, both fungi or neither species as described in the General Methods. Fumigation reduces populations of soil-borne organisms, reducing fungal infection of roots, especially early in the growing season. This chemical treatment was undertaken to allow *M. bolleyi* and *B. sorokiniana* inoculum to become established in the absence of other fungi, and so that the effects of inoculum could be more readily observed. Inoculum was also applied to non-fumigated plots. Wheat varieties were chosen on the basis of their reaction to *B. sorokiniana*.

8.3.2 METHODS

Fumigation and inoculation treatments are described in the General Methods, as are sowing dates and all agronomic methods employed in field experiments.

Four varieties of wheat with different reactions to *B. sorokiniana* were sown in a factorially designed experiment in 1988:

Oxley - moderately susceptible

Machete - susceptible

Dagger - moderately resistant

Kite - moderately resistant (J. Lewis and A. J. Rathjen, personal communication; Dubé and Brooks, 1986; Wallwork, 1989; P. J. L. Whittle, personal communication). The experiment consisted of four varieties and four inoculation treatments, on both

fumigated and non-fumigated plots (a total of eight treatments), which were replicated eight times at both Mannum and Sanderston.

A further trial was sown in 1989, using higher inoculum levels than those employed in 1988, including two levels of *M. bolleyi* inoculum. Two wheat varieties (Kite and Machete) were sown, both replicated ten times for each treatment at Mannum and Caloote. This experiment consisted of six inoculation treatments on both fumigated and non-fumigated plots (a total of twelve treatments).

Sampling

1988 - Seedling emergence was recorded at Mannum and Sanderston on June 29 and July 2, respectively. The number of seedlings per plot row was counted in the centre two rows of six replicates of all eight treatments for the four wheat varieties.

Ten plants were carefully dug from each plot on June 21, July 12, August 2 and September 5. Plants were stored in plastic bags at 5°C until processing. Soil was washed from the roots under running tapwater. Disease ratings (based on the incidence and severity of subcrown internode lesions) were calculated, except for the June sampling when subcrown internodes were still obscured by the coleoptile. All subcrown internodes were plated on isolation medium and infection levels determined. At each sample date, twelve 1.0 cm long seminal root segments were selected at random, from each set of ten plants, and plated on isolation medium. Crown roots rather than seminal roots were plated in September, because the soil had hardened to such an extent that seminal roots could not be easily extracted from the soil. Shoots were dried and weighed at each sample date.

Plots were harvested on November 18, when six replicates per site were sampled. Whole plants were removed, by hand, from two rows of each plot (which had not been sampled during the growing season). The following parameters were determined:

number of plants per 1.2 m plot row

number of fertile tillers per plant

total shoot weight per plant.

Finally, plants were threshed, and grain yield per plant measured.

Data are presented over all varieties, pooled for each treatment, as there were no consistent significant differences between varieties. This effectively increases replication.

1989 - Ten plants were sampled from each plot on July 17 and August 21, when disease rating values for subcrown internodes were calculated, root segments and subcrown internodes plated on isolation medium and shoots dried and weighed. All methods were identical to those described above for the 1988 field experiments.

In early December, whole plants were removed from the two centre rows (which had not been sampled during the season) of five replicates at each site. All subcrown internodes from a twenty plant sub-sample from each replicate were rated for *B. sorokiniana* infection, as described in the General Methods, and the weight of crown roots on this twenty plant sub-sample was also measured. Plant weight per 1.2 m plot row was determined, and plants were then threshed to measure grain yields. Protein analyses were carried out on grain samples from three replicates of each treatment from the Caloote site, and three replicates of the uninoculated fumigated and unfumigated plots from the Mannum site. Grain samples of 50 g were milled and an infralyzer used to determine the protein content at 11% moisture.

8.3.3 RESULTS

8.3.3.1 1988 Field Experiments

Shoot Dry Weight

Overall, plants from fumigated plots had a higher shoot weight than those from non-fumigated plots. This was significant ($P<0.05$) at Mannum in July, August, September and November, when plants from fumigated areas weighed 32%, 53%, 59% and 41% more, respectively, than those from non-fumigated plots (Table 8.13). Plants on fumigated areas at Sanderston weighed up to 26% more than those from unfumigated areas, but this was only significant ($P<0.05$) in August and September.

Fumigation led to a higher shoot weight at Mannum than at Sanderston, but by

September this difference was slight, and there was no difference between sites by November (Table 8.13). Untreated plots at Mannum produced more shoot material than those at Sanderston between June and August, but the situation was reversed in September and November.

TABLE 8.13: Dry shoot weight (mg/plant) of wheat plants at Sanderston (S'ton) and Mannum (M'M) in June, July, August, September and November, 1988. Plots were inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs), both (MbBs) or neither on areas treated with methyl bromide soil fumigant (F) and on untreated areas (NF). (Values are the mean of three replicates in June, seven in July and August, twelve in September and 23 in November).

	JUNE		JULY		AUGUST		SEPTEMBER		NOVEMBER	
Treatment	**S'ton**	**M'M**	**S'ton**	**M'M**	**S'ton**	**M'M**	**S'ton**	**M'M**	**S'ton**	**M'M**
Mb-F	12.0	17.9	43.7	103.2	306.1	596.5	2021.8	3628.3	4966.5	4596.1
Bs-F	15.9	20.5	56.4	86.2	307.1	646.1	1970.0	2039.3	4563.5	4641.3
MbBs-F	14.0	21.6	42.2	92.3	312.6	559.2	2005.0	2053.3	4977.0	4541.7
F	13.8	17.5	45.3	105.7	315.2	524.2	1955.8	2001.7	4673.5	4651.7
Mean	13.9	19.4	46.9	96.9	310.3	581.5	1988.2	2430.7	4795.1	4607.7
Mb-NF	10.3	20.3	38.4	66.3	247.4	268.2	1579.4	978.7	4594.8	2674.8
Bs-NF	11.4	17.1	39.1	66.3	238.5	253.9	1308.8	844.2	4804.8	2659.6
MbBs-NF	12.8	21.3	40.3	65.9	268.9	296.5	1468.3	1097.5	5068.3	2622.2
NF	13.7	20.8	40.8	64.8	238.5	276.1	1559.8	1070.0	4660.9	2903.9
Mean	12.1	19.9	39.7	65.8	248.3	273.7	1479.1	997.6	4782.2	2715.1
LSD(0.05) Treatment	3.9	4.0	9.4	19.0	76.9	97.2	448.3	1813.4	540.6	600.0
LSD(0.05) Means	3.0	4.8	10.9	15.3	22.6	105.3	186.3	1304.3	415.2	160.3

Plants on uninoculated plots from fumigated and unfumigated areas often produced a greater shoot mass than those on the corresponding inoculated plots, but not significantly (Table 8.13). At Mannum in July, plants from fumigated plots inoculated with *B. sorokiniana* weighed 18% less than those from uninoculated plots. This was statistically significant ($P<0.05$). Shoot weight of plants from fumigated plots inoculated with *M. bolleyi* was unusually high at Mannum in September, undoubtedly due to some experimental error.

Plants inoculated with *M. bolleyi* + *B. sorokiniana* tended to have a greater shoot weight than those inoculated with either species alone, especially on non-fumigated areas

(Table 8.13). However, the only significant ($P<0.05$) difference between these treatments was at Mannum in June, when plants on non-fumigated areas inoculated with *M. bolleyi* + *B. sorokiniana* weighed 20% more than those inoculated with *B. sorokiniana* alone.

Disease Rating

The disease rating increased with successive sample dates, as infection levels on the subcrown internodes increased. Overall, fumigation reduced disease rating at Sanderston and Mannum in July by 29% and 40%, respectively, but neither was significant (Table 8.14). Fumigation did not reduce the disease rating recorded at Sanderston in August or September: disease rating was in fact significantly higher on fumigated than on non-fumigated plots in August. However, fumigation significantly ($P<0.05$) decreased disease rating at Mannum in August and September, by 70% and 32%, respectively. Disease rating on non-fumigated plots at Mannum was higher than that at Sanderston, which was reflected by the higher frequency with which *B. sorokiniana* was isolated at the Mannum site (Figures 8.1 - 8.7), especially later in the growing season.

There were few significant differences between inoculation treatments at either site in regard to disease rating values (Table 8.14). Inoculation with both fungi reduced the disease rating recorded at Mannum in July by 22% from that of plants inoculated with *B. sorokiniana* alone, but this was not statistically significant. On unfumigated areas at Sanderston, inoculation with *M. bolleyi* + *B. sorokiniana* reduced disease rating in August and September by 34% and 55%, respectively, from that of plants inoculated with *B. sorokiniana* alone. However, this was only statistically significant ($P<0.05$) at Sanderston in September. Plants inoculated with *B. sorokiniana* had a disease rating up to 72% greater than those inoculated with *M. bolleyi*, particularly on the non-fumigated areas, but these differences were not significant.

Seedling Emergence

Seedling emergence was higher at Mannum than at Sanderston (Table 8.15). Fumigation led to 10% more seedlings/row at Sanderston, but was associated with a

slightly reduced emergence at Mannum. Differences between means revealed no significant increase in emergence due to fumigation.

TABLE 8.14: Disease rating (%) on wheat subcrown internodes at Sanderston (S'ton) and Mannum in July, August and September, 1988. Inoculation treatments are described in Table 8.13. (Values are the mean of ten replicates in July, eight in August and twelve in September). Values in brackets are $\sqrt{(x+0.5)}$ transformed means for July and August, and $\sqrt{x}$ means for September. The LSD (0.05) is based on these transformations.

	JULY				AUGUST				SEPTEMBER			
Treatment	**S'ton**		**Mannum**		**S'ton**		**Mannum**		**S'ton**		**Mannum**	
Mb-F	0.8	(1.1)	1.9	(1.4)	4.7	(1.7)	1.8	(1.3)	14.3	(3.2)	9.3	(2.4)
Bs-F	0.8	(1.0)	0	(0.7)	5.5	(2.2)	1.8	(1.3)	14.5	(3.6)	11.8	(3.0)
MbBs-F	0	(0.7)	0	(0.7)	8.5	(2.9)	1.8	(1.3)	21.2	(4.5)	12.0	(3.0)
F	0.5	(0.9)	0.4	(0.9)	9.5	(2.2)	2.0	(1.4)	9.1	(2.9)	10.3	(3.0)
Mean	0.5	(0.9)	0.6	(0.9)	7.1	(2.3)	1.9	(1.3)	14.8	(3.6)	10.9	(2.9)
Mb-NF	1.0	(0.7)	0.7	(1.0)	0.7	(1.0)	6.3	(2.4)	10.3	(2.9)	17.0	(3.9)
Bs-NF	0	(0.7)	1.6	(1.4)	2.5	(1.5)	6.2	(2.5)	13.4	(3.3)	12.2	(3.3)
MbBs-NF	1.4	(1.1)	1.3	(1.2)	1.7	(1.3)	7.1	(2.5)	6.1	(1.7)	20.6	(4.2)
NF	0.4	(0.7)	0.4	(0.9)	0.3	(0.8)	5.4	(2.3)	17.0	(3.3)	14.1	(3.7)
Mean	0.7	(0.8)	1.0	(1.1)	1.3	(1.2)	6.3	(2.4)	11.7	(2.8)	16.0	(3.8)
LSD(0.05) Treatment		0.5		0.5		1.3		0.8		1.5		1.3
LSD(0.05) Means		0.6		0.8		0.7		0.2		2.2		0.8

Inoculation with *M. bolleyi* alone reduced emergence by only 3 - 16%, while *B. sorokiniana* alone reduced emergence by up to 13%. This was significant ($P<0.05$) at Mannum (Table 8.15). Inoculation with both species of fungi had a less deleterious effect on seedling emergence than did *M. bolleyi* or *B. sorokiniana* alone, although the difference was not statistically significant. *M. bolleyi* + *B. sorokiniana* reduced seedling numbers by only 6 - 8%.

Number of Plants per Row

In every case, there were fewer plants per plot row at maturity in November than in late June-early July when seedling emergence was recorded (Table 8.15). This

difference was significant (P<0.05) on fumigated and non-fumigated plots at both sites. Fumigated plots had fewer plants per row in November than they did at emergence: 26% fewer at Sanderston and 21% fewer at Mannum. This was also true of non-fumigated plots, which had 23% fewer plants at Sanderston and 34% fewer at Mannum by November. Fumigation partly alleviated the incidence of plant death at Mannum, but not to the same extent at Sanderston. The time of seedling death during the growing season was not determined.

Plots at Mannum had more plants per row at maturity than those at Sanderston, particularly on the fumigated areas (Table 8.15). Overall, fumigation significantly (P<0.05) increased the number of plants/row at Mannum but not at Sanderston.

TABLE 8.15: Seedling emergence (plants/1.2 m plot row), number of plants per 1.2 m plot row in November, number of fertile tillers/plant at maturity and grain yield (g/plant) of wheat plants at Sanderston (S'ton) and Mannum (M'num), 1988. Inoculation treatments are described in Table 8.13. (Values are the mean of 24 replicates for each treatment).

	EMERGENCE (Plants/Row)		PLANTS/ROW (NOVEMBER)		FERTILE TILLERS/PLANT		GRAIN YIELD (g/plant)	
Treatment	S'ton	M'num	S'ton	M'num	S'ton	M'num	S'ton	M'num
Mb-F	39.1	44.9	30.2	36.6	2.3	2.0	1.3	1.5
Bs-F	46.6	46.7	35.5	38.1	2.2	2.1	1.1	1.5
MbBs-F	43.9	48.5	31.9	38.7	2.3	2.0	1.3	1.5
F	46.8	46.1	32.9	34.1	2.2	2.1	1.2	1.5
Mean	44.1	46.6	32.6	36.9	2.3	2.1	1.2	1.5
Mb-NF	41.9	49.3	31.0	32.4	2.0	1.4	1.2	0.9
Bs-NF	40.0	44.9	29.1	32.8	2.1	1.4	1.3	0.8
MbBs-NF	39.8	47.2	28.8	32.4	2.3	1.4	1.3	0.9
NF	36.8	51.6	33.3	30.2	2.0	1.4	1.2	1.0
Mean	39.6	48.3	30.6	32.0	2.1	1.4	1.3	0.9
LSD Treatment	5.2	5.3	4.3	4.4	0.2	0.2	0.2	0.2
LSD Means	8.6	6.0	5.4	1.7	0.2	0.1	0.2	0.1
LSD (0.05)	Emergence vs. Plants/Row				S'ton = 2.6; Mannum = 3.8			

Inoculation had little effect on the number of plants per row. Uninoculated, non-fumigated plots had significantly (P<0.05) more plants per row at Sanderston than those inoculated with *M. bolleyi* + *B. sorokiniana*. At Mannum, fumigated plots inoculated

with *B. sorokiniana* or with *M. bolleyi* + *B. sorokiniana* had significantly (P<0.05) more plants per row than all the non-fumigated plots.

Number of Fertile Tillers per Plant

Plants at Sanderston produced more fertile tillers than those at Mannum, especially on the non-fumigated areas (Table 8.15). Overall, fumigation significantly (P<0.05) enhanced tillering at both sites, by 9% at Sanderston and by 33% at Mannum. Inoculation treatments had little effect on the number of fertile tillers/plant.

Grain Yield

Overall, fumigation significantly (P<0.05) enhanced yield at Mannum, but not at Sanderston (Table 8.15). Fumigation increased yield at Mannum by 40%, but actually reduced yield at Sanderston. Inoculation treatments had little effect on grain yield.

Isolation of Microdochium bolleyi and Bipolaris sorokiniana

Graphs of percentage isolation frequencies are presented (Figures 8.1 - 8.7), but statistical analyses were performed on transformed data. The corresponding transformed data are in Appendix A.

Infection levels were low in June (Figure 8.1) and July (Figures 8.2, 8.3), but increased as the growing season progressed. In June, July and August seminal roots of plants on non-fumigated areas inoculated with *M. bolleyi* at Sanderston were infected with more *M. bolleyi* than were plants on the uninoculated plots, but this was not significant (Figures 8.1, 8.2, 8.4). Inoculation enhanced infection of seminal roots with *M. bolleyi* at Mannum in August (Figure 8.4), and this was also significant (P<0.05) for the subcrown internodes from fumigated plots at Sanderston (Figure 8.5). In September, crown roots of plants at Sanderston on fumigated areas inoculated with *M. bolleyi* were infected by significantly (P<0.05) more *M. bolleyi* than crown roots of uninoculated plants (Figure 8.6).

There was little evidence that inoculation with *B. sorokiniana* was effective in increasing the incidence of *B. sorokiniana*. Many plots, particularly early in the growing

season, were not infected with *B. sorokiniana*, even those that had been inoculated. At Sanderston in September, subcrown internodes from fumigated plots inoculated with *B. sorokiniana* were infected with significantly ($P<0.05$) more *B. sorokiniana* than were subcrown internodes of uninoculated plants (Figure 8.7). At Mannum, the seminal roots in August (Figure 8.4) and the crown roots in September (Figure 8.6) from plots inoculated with *B. sorokiniana* were infected more by *B. sorokiniana* than were the uninoculated plants on non-fumigated areas.

The frequency of *B. sorokiniana* isolated from plants was much lower than the incidence of *M. bolleyi*, even on the subcrown internodes of inoculated plants (Figures 8.1 - 8.7). Infection levels were generally higher on non-fumigated than on fumigated plots, but this was rarely significant. At Mannum in August, seminal roots from fumigated plots inoculated with either *M. bolleyi* or with *B. sorokiniana* were infected significantly ($P<0.05$) less than seminal roots from the corresponding non-fumigated plots (Figure 8.4).

Plants inoculated with *M. bolleyi* + *B. sorokiniana* were sometimes infected with less *M. bolleyi* or *B. sorokiniana* than those from plots inoculated with either species of fungus alone, particularly on the non-fumigated areas (Figures 8.1 - 8.7). However, these differences between treatments were not always significant.

In June, seminal roots from plots inoculated with *M. bolleyi* + *B. sorokiniana* were infected with 50% less *B. sorokiniana* than those inoculated with *B. sorokiniana* alone (Figure 8.1). This occurred on non-fumigated plots at Sanderston, but was not significant. This was not evident in either July (Figure 8.2) or August (Figure 8.4) but, in September (Figure 8.6) on the non-fumigated plots at Mannum, crown roots from plots inoculated with *M. bolleyi* + *B. sorokiniana* were infected with 25% less *B. sorokiniana* than were those from plots inoculated with *B. sorokiniana* alone, but this was not significant. Also in September, at both sites, crown roots from fumigated plots inoculated with *M. bolleyi* + *B. sorokiniana* were not infected with any *B. sorokiniana*, while those from plots inoculated with *B. sorokiniana* alone were colonised by this species (Figure 8.6).

In July, inoculation with *M. bolleyi* + *B. sorokiniana* had no effect on the rate of

FIGURE 8.1: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of wheat plants at Mannum and Sanderston in June, 1988. Plots were inoculated with *M. bolleyi* (Mb), *B. sorokiniana* (Bs), both (MbBs) or neither on areas treated with methyl bromide soil fumigant (F) and on untreated areas (NF). (Values are the mean of four replicates for each treatment).

FIGURE 8.2: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of wheat plants at Mannum and Sanderston in July, 1988. Inoculation treatments are described in Figure 8.1. (Values are the mean of seven replicates for each treatment).

FIGURE 8.3: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from subcrown internodes of wheat plants at Mannum and Sanderston in July, 1988. Inoculation treatments are described in Figure 8.1. (Values are the mean of nine replicates for each treatment).

Figure 8.1

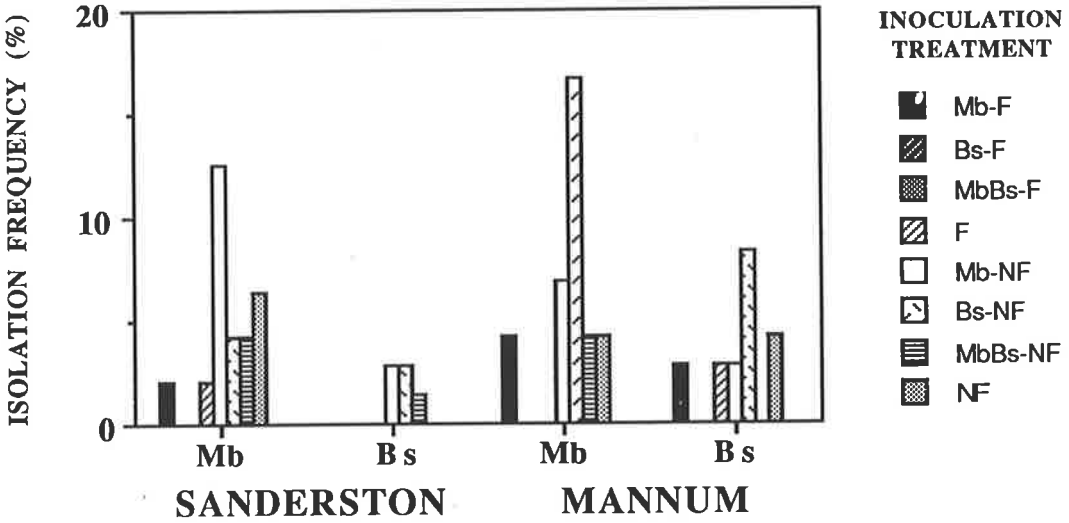


Figure 8.2

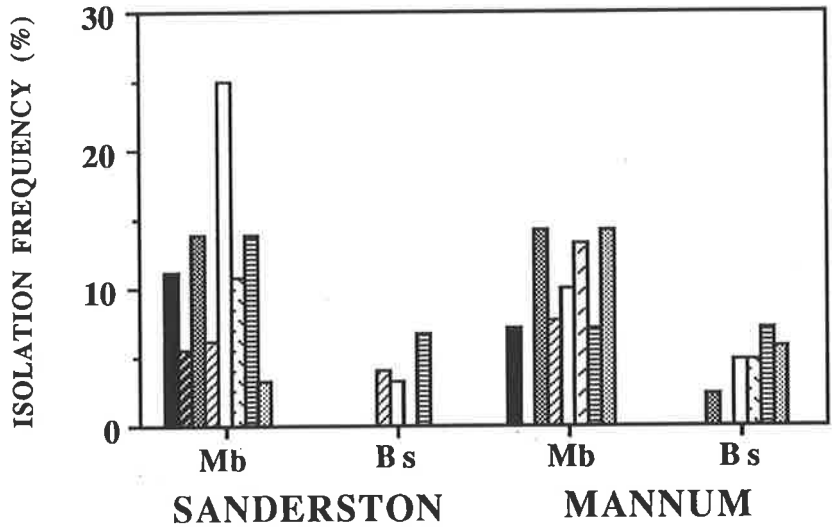


Figure 8.3

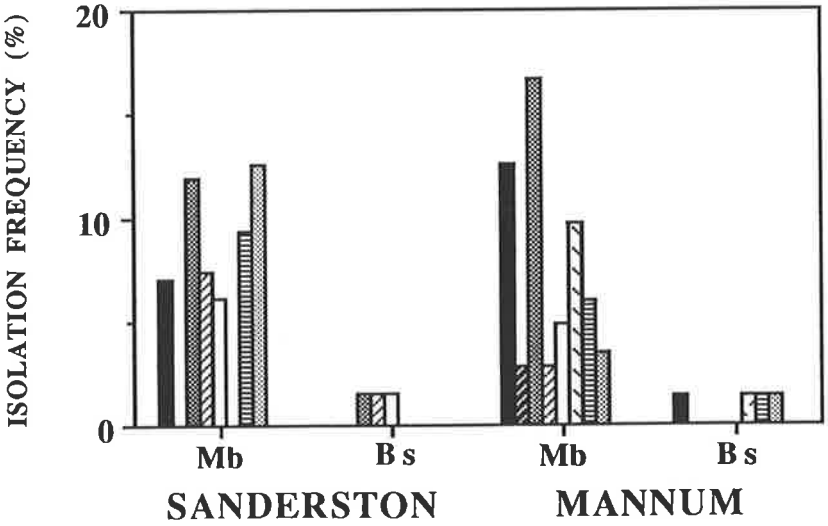


FIGURE 8.4: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of wheat plants at Mannum and Sanderston in August, 1988. Inoculation treatments are described in Figure 8.1. (Values are the mean of eight replicates for each treatment).

FIGURE 8.5: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from subcrown internodes of wheat plants at Mannum and Sanderston in August, 1988. Inoculation treatments are described in Figure 8.1. (Values are the mean of eight replicates for each treatment).

FIGURE 8.6: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from crown roots of wheat plants at Mannum and Sanderston in September, 1988. Inoculation treatments are described in Figure 8.1. (Values are the mean of eight replicates for each treatment).

Figure 8.4

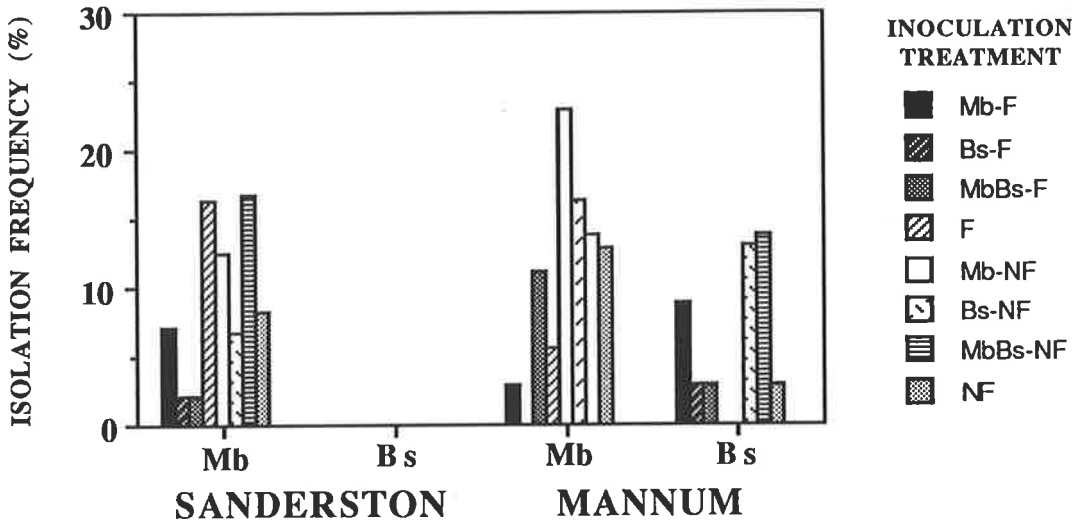


Figure 8.5

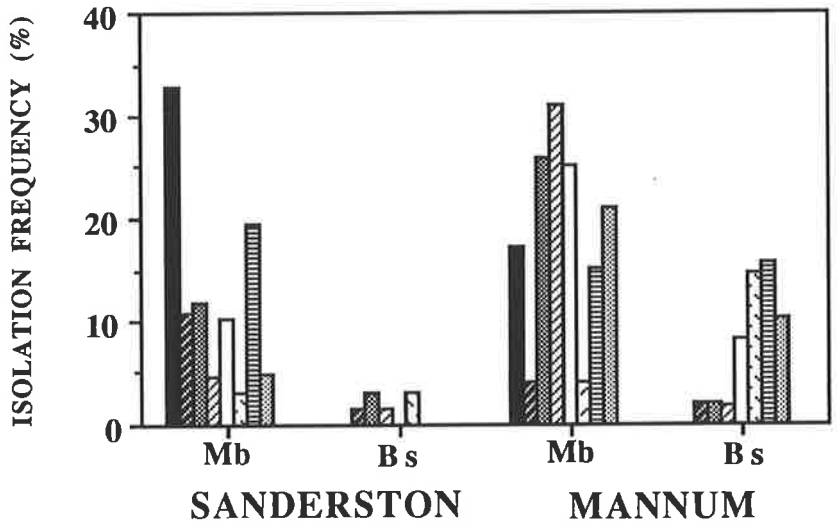


Figure 8.6

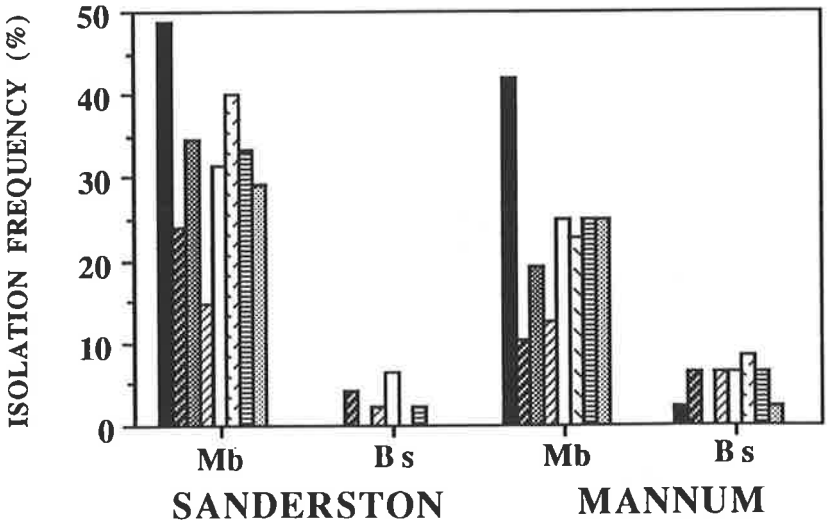
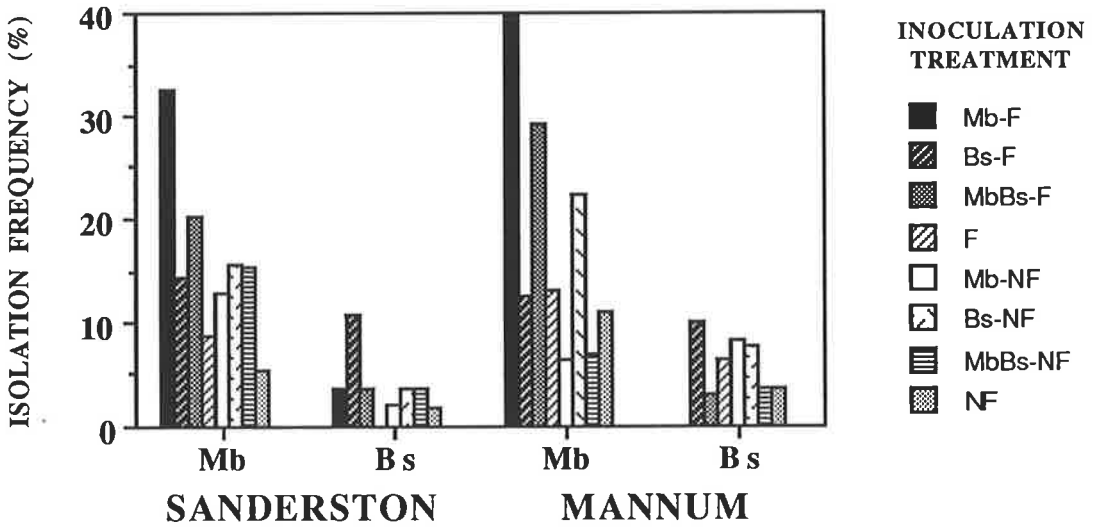


FIGURE 8.7: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from subcrown internodes of wheat plants at Mannum and Sanderston in September, 1988. Inoculation treatments are described in Figure 8.1. (Values are the mean of eight replicates for each treatment).

Figure 8.7



B. sorokiniana recovered from subcrown internodes (Figure 8.3). Subcrown internodes from non-fumigated plots at Sanderston sampled in August (Figure 8.5) were not infected with *B. sorokiniana* where *M. bolleyi* + *B. sorokiniana* inoculum was applied, but this species infected subcrown internodes where *B. sorokiniana* alone was added to the soil. *B. sorokiniana* infection of subcrown internodes in September (Figure 8.7) was reduced by up to 68% where *M. bolleyi* + *B. sorokiniana* were inoculated into the soil, compared to plots inoculated with *B. sorokiniana* alone. However, this was not statistically significant, and there was no difference between *B. sorokiniana* and *M. bolleyi* + *B. sorokiniana* treatments on non-fumigated plots at Sanderston.

8.3.3.2 1989 Field Experiments

Shoot Dry Weight

Fumigation enhanced shoot dry weight (based on overall means) of both varieties at both sites. In July (Table 8.16), these differences were statistically significant ($P<0.05$) for all but Kite at Mannum, when fumigation increased shoot weight of Kite by 15 - 16% and that of Machete by 21 - 22%. The difference between overall means was significant ($P<0.05$) in August (Table 8.17), when fumigation increased shoot weight of Kite by 41 - 57% and that of Machete by 37 - 52%. In December (Table 8.18), differences between overall means were significant ($P<0.05$) at both sites, and fumigation enhanced shoot weight of Kite by 15 - 41% and that of Machete by 11 - 35%. In both August (Table 8.17) and December (Table 8.18), fumigation increased shoot weight at Caloote to a greater extent than at Mannum, whereas there was little difference between sites in July (Table 8.16).

Inoculation with *B. sorokiniana* did not necessarily result in the shoots of Machete (susceptible) weighing less than those of Kite (moderately resistant). Inoculation with *B. sorokiniana* did not reduce the shoot weight of Kite at either site on any sample date. At Caloote, weight of Machete was only reduced in July (by 5%), but this was not significant (Table 8.16). Reductions in Machete shoot weight were not significant at Mannum, but a reduction of 4 - 13% was recorded between July and December (Tables

8.16 - 8.18).

Inoculation with the high rate of *M. bolleyi* did not result in a significantly different shoot weight to inoculation with the lower level of *M. bolleyi*. The only exception was on fumigated plots of Machete at Mannum in August (Table 8.17), when plants inoculated with the high level of *M. bolleyi* weighed 36% less than those inoculated with the low rate of *M. bolleyi*, and this was significant ($P<0.05$). Similarly, there was no significant difference in shoot weight between plants inoculated with either level of *M. bolleyi* in conjunction with *B. sorokiniana*.

TABLE 8.16: Dry shoot weight (mg/plant) of Kite and Machete wheat at Caloote and Mannum in July, 1989. Plots on areas treated with methyl bromide soil fumigant (F), or on adjacent untreated areas (NF), were inoculated as follows: *Microdochium bolleyi* was applied at low (Mb1) and high (Mb2) rates, alone and in conjunction with *Bipolaris sorokiniana* (Mb1Bs and Mb2Bs); *B. sorokiniana* (Bs) was applied at one inoculum level only. Control plots (F and NF) were inoculated with neither fungus. (Values are the mean of four replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	59.4	67.9	67.2	87.5
Mb2-F	60.7	55.8	98.3	86.8
Bs-F	71.1	74.8	67.0	93.8
Mb1Bs-F	62.2	73.9	80.7	77.5
Mb2Bs-F	49.3	56.1	78.7	82.0
F	63.2	71.9	89.9	98.1
Mean	61.0	66.7	80.3	87.6
Mb1-NF	43.5	63.0	54.5	63.0
Mb2-NF	43.4	55.9	69.7	80.9
Bs-NF	60.1	48.2	76.9	60.4
Mb1Bs-NF	49.7	44.1	83.7	66.1
Mb2Bs-NF	56.0	41.6	64.2	81.9
NF	56.7	57.8	55.2	63.1
Mean	51.6	51.8	67.4	69.2
LSD Treatments	21.2	23.6	24.6	28.1
LSD Means	9.2	12.3	18.2	15.4

Inoculation with either *M. bolleyi* or *B. sorokiniana* alone had little effect on the shoot weight of plants. *M. bolleyi* alone did not significantly reduce shoot weight in July

(Table 8.16), nor did inoculation with *B. sorokiniana*. In August, the same was true, except at Mannum where shoot weight of Machete on fumigated plots was significantly ($P<0.05$) decreased by the high level of *M. bolleyi* inoculum (Table 8.17). Neither *M. bolleyi* nor *B. sorokiniana* significantly reduced shoot weight of either variety in December (Table 8.18), except on fumigated areas at Mannum: *B. sorokiniana* and the high rate of *M. bolleyi* reduced shoot weight of Machete at maturity by 20% and 23%, respectively. These reductions were both significant ($P<0.05$).

TABLE 8.17: Dry shoot weight (mg/plant) of Kite and Machete wheat at Caloote and Mannum in August, 1989. Inoculation treatments are described in Table 8.16. (Values are the mean of three replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	846.6	919.8	1205.0	1397.5
Mb2-F	707.2	874.7	1278.2	889.3
Bs-F	954.0	831.7	1383.3	1304.8
Mb1Bs-F	816.4	767.9	1038.2	967.2
Mb2Bs-F	896.3	746.3	966.3	1269.3
F	686.8	780.6	1089.3	1315.5
Mean	817.9	820.2	1160.1	1190.6
Mb1-NF	277.8	550.6	912.6	748.3
Mb2-NF	262.2	461.7	609.4	1038.3
Bs-NF	467.9	390.0	753.0	586.5
Mb1Bs-NF	319.9	215.8	650.8	672.2
Mb2Bs-NF	418.9	302.2	534.0	788.0
NF	386.7	417.4	661.2	662.3
Mean	355.6	389.6	686.8	749.3
LSD Treatments	306.3	285.3	334.5	379.1
LSD Means	93.8	72.2	153.3	343.7

There were some differences in shoot weight between plants inoculated with *M. bolleyi* and those inoculated with *B. sorokiniana*. Plots inoculated with *B. sorokiniana* generally produced less shoot material than those inoculated with *M. bolleyi*. In July, this was significant ($P<0.05$) only at Mannum, when shoots of Kite on fumigated plots inoculated with *B. sorokiniana* weighed 32% less than those inoculated with the high rate of *M. bolleyi* (Table 8.16). In August, shoots of plants inoculated with *B. sorokiniana*

weighed up to 44% less than those inoculated with *M. bolleyi*, but these differences were rarely significant (Table 8.17), and shoots of Kite inoculated with *B. sorokiniana* often weighed more than those inoculated with *M. bolleyi*. Nor was this significant in December, when plants inoculated with *M. bolleyi* weighed only 2 - 11% more than those inoculated with *B. sorokiniana* alone (Table 8.18), and plants inoculated with *B. sorokiniana* sometimes weighed more than those inoculated with *M. bolleyi*.

TABLE 8.18: Dry shoot weight (g/1.2 m plot row) of Kite and Machete wheat at Caloote and Mannum in December, 1989. Inoculation treatments are described in Table 8.16. (Values are the mean of five replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	214.7	220.6	264.5	259.1
Mb2-F	201.7	214.5	250.6	220.4
Bs-F	222.5	229.9	259.4	231.7
Mb1Bs-F	236.5	232.6	254.0	232.0
Mb2Bs-F	227.9	236.6	234.4	244.5
F	226.4	226.5	236.5	287.9
Mean	221.6	226.8	249.9	245.9
Mb1-NF	129.8	160.5	219.7	214.2
Mb2-NF	129.6	149.2	209.9	209.2
Bs-NF	144.5	143.4	208.2	223.0
Mb1Bs-NF	106.3	128.1	217.2	194.1
Mb2Bs-NF	138.1	165.4	198.6	231.7
NF	139.3	131.8	223.8	237.1
Mean	131.3	146.4	212.9	218.2
LSD Treatments	34.2	38.8	49.2	54.9
LSD Means	21.6	18.5	13.8	19.9

Shoot weight of plants inoculated with both fungi was sometimes greater than that of plants inoculated with *B. sorokiniana*, and this occurred more frequently at Mannum than at Caloote. However, weight of plants inoculated with *B. sorokiniana*, particularly in July and August (Tables 8.16, 8.17), was greater than that of plants inoculated with both fungi, especially at Caloote. In July, inoculation with *M. bolleyi* + *B. sorokiniana* led to an 8 - 26% increase in shoot weight over that of plants inoculated with *B. sorokiniana* alone, which was not significant (Table 8.16), and some plants inoculated with *B.*

sorokiniana weighed up to 31% more than those inoculated with *M. bolleyi* + *B. sorokiniana*. These differences were not significant in either August (Table 8.17) or December (Table 8.18), when some plants inoculated with *M. bolleyi* + *B. sorokiniana* weighed 13 - 26% and 1 - 13% more, respectively, than those inoculated with *B. sorokiniana* alone. Conversely, many plants inoculated with *B. sorokiniana* weighed up to 45% and 26% more in August and December, respectively, than those inoculated with *M. bolleyi* + *B. sorokiniana*. There was no evidence to suggest that the higher rate of *M. bolleyi* inoculum reduced the effect of *B. sorokiniana* on shoot growth to a greater extent than did the lower rate of *M. bolleyi*.

Disease Rating

There was little difference in overall disease rating values between varieties, especially in December (Table 8.21). However, on non-fumigated plots in July, Kite had a higher disease rating than Machete at Caloote, but the reverse was true at Mannum (Table 8.19). In August, Kite had a higher disease rating than Machete on non-fumigated plots at both sites (Table 8.20). There was little difference between varieties in December (Table 8.21), although the disease rating of Kite was marginally greater than that of Machete on non-fumigated plots.

Overall, fumigation did not reduce disease rating values. In most cases, disease rating was actually higher on fumigated than on non-fumigated plots, and some of these differences were significant ($P<0.05$) in July (Table 8.19) and August (Table 8.20). There was no significant difference between overall means of fumigated and non-fumigated plots in December (Table 8.21).

Inoculation with *B. sorokiniana* did not necessarily result in Machete having a higher disease rating than Kite for all samples. Machete inoculated with *B. sorokiniana* had a disease rating up to 60% higher than Kite in July (Table 8.19), but Kite and Machete had the same disease rating on non-fumigated plots at Mannum. However, in August (Table 8.20), Machete and Kite had similar disease ratings at Caloote, and the disease rating of Kite was greater than that of Machete at Mannum. At Mannum, Machete inoculated with *B. sorokiniana* had a disease rating 38% higher than that of Kite on

fumigated areas in December (Table 8.21), while there was little difference between varieties on non-fumigated soil.

TABLE 8.19: Disease rating (%) on subcrown internodes of Kite and Machete wheat at Caloote and Mannum in July, 1989. Inoculation treatments are described in Table 8.16. (Values are the mean of four replicates for each treatment). Values in brackets are $\sqrt{(x+0.5)}$ transformed means, and the LSD (0.05) is based on these transformations.

	CALOOTE				MANNUM			
Treatment	**Kite**		**Machete**		**Kite**		**Machete**	
Mb1-F	7.8	(2.6)	10.6	(3.3)	10.0	(2.6)	7.5	(2.7)
Mb2-F	2.6	(1.7)	4.6	(2.1)	7.5	(2.5)	6.3	(2.3)
Bs-F	6.6	(2.5)	10.3	(3.1)	5.7	(2.4)	14.1	(3.8)
Mb1Bs-F	8.1	(2.9)	5.8	(2.3)	11.1	(3.3)	13.8	(3.4)
Mb2Bs-F	6.3	(2.3)	4.7	(2.1)	11.3	(2.8)	7.5	(2.7)
F	3.1	(1.7)	11.7	(3.3)	10.6	(3.2)	6.7	(2.5)
Mean	5.8	(2.3)	8.0	(2.7)	9.4	(2.8)	9.3	(2.9)
Mb1-NF	3.2	(1.8)	4.1	(1.9)	5.2	(2.2)	4.5	(2.2)
Mb2-NF	4.7	(1.9)	4.5	(2.0)	3.5	(1.7)	9.4	(2.7)
Bs-NF	1.9	(1.4)	4.3	(2.2)	5.6	(2.2)	5.5	(2.4)
Mb1Bs-NF	7.6	(2.6)	0	(0.7)	5.0	(2.1)	0	(0.7)
Mb2Bs-NF	0.7	(1.0)	0.6	(1.0)	4.4	(2.1)	5.0	(2.3)
NF	3.9	(1.9)	3.0	(1.6)	5.5	(2.3)	8.8	(2.8)
Mean	3.7	(1.8)	2.8	(1.6)	4.9	(2.1)	5.5	(2.2)
LSD Treatments		1.5		1.3		2.0		1.6
LSD Means		0.7		0.6		0.4		1.2

Most plots inoculated with *B. sorokiniana* had a higher disease rating than those inoculated with *M. bolleyi*, although this was never statistically significant, and plants inoculated with *B. sorokiniana* sometimes had a disease rating lower than that of those inoculated with *M. bolleyi*, especially in December (Table 8.21). In July, the disease rating of plants inoculated with *B. sorokiniana* was up to 53% higher than those inoculated with *M. bolleyi* (Table 8.19), but many plots inoculated with *B. sorokiniana* had a disease rating lower than those inoculated with *M. bolleyi*. Addition of *B. sorokiniana* inoculum increased disease rating by up to 34% in August (Table 8.20), but by only 7 - 24% in December (Table 8.21). However, disease rating was not always increased by inoculation with *B. sorokiniana*.

Many plots inoculated with *M. bolleyi* + *B. sorokiniana* had a lower disease rating than those inoculated with *B. sorokiniana* alone, although plots inoculated with both fungi often had a disease rating greater than that of those inoculated with only *B. sorokiniana* in August (Table 8.20) and December (Table 8.21). In July, inoculation with both species resulted in a disease rating lower than that produced by inoculation with *B. sorokiniana* alone (Table 8.19), and this was significant ($P<0.05$) for Machete at both sites. However, inoculating Kite with *M. bolleyi* + *B. sorokiniana* resulted in a disease rating higher than that of plants inoculated with *B. sorokiniana* alone. Differences between treatment with *M. bolleyi* + *B. sorokiniana* and *B. sorokiniana* alone were not significant in August (Table 8.20). By December (Table 8.21), fewer plots inoculated with *B. sorokiniana* had a higher disease rating than those inoculated with *M. bolleyi* + *B. sorokiniana*. This was only significant ($P<0.05$) for Machete at Mannum on fumigated areas, where disease rating was 25% less when plants were inoculated with both species of fungus. In contrast, inoculation with *M. bolleyi* + *B. sorokiniana* at Caloote led to a higher disease rating in some cases than did inoculation with *B. sorokiniana* alone.

TABLE 8.20: Disease rating (%) on subcrown internodes of Kite and Machete wheat at Caloote and Mannum in August, 1989. Inoculation treatments are described in Table 8.16. (Values are the mean of three replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	12.6	29.3	39.3	30.2
Mb2-F	32.0	21.0	23.7	32.2
Bs-F	30.0	34.8	40.0	34.0
Mb1Bs-F	19.3	38.3	35.9	33.4
Mb2Bs-F	33.3	41.6	26.4	43.6
F	19.8	40.2	43.9	23.5
Mean	24.5	34.2	34.9	32.8
Mb1-NF	18.8	18.2	32.5	24.7
Mb2-NF	14.4	6.7	33.7	21.0
Bs-NF	18.5	13.1	37.3	17.8
Mb1Bs-NF	18.5	10.0	30.5	32.3
Mb2Bs-NF	23.6	11.1	29.2	30.2
NF	11.4	14.1	25.3	30.7
Mean	17.5	12.2	31.4	26.1
LSD Treatments	18.9	22.9	24.0	21.6
LSD Means	8.8	8.2	10.1	9.2

There was no significant difference in disease rating between plants inoculated with the low level of *M. bolleyi* and those inoculated with the high rate of *M. bolleyi* (Tables 8.19 - 8.21), either alone or in conjunction with *B. sorokiniana*. However, plants inoculated with the low level of *M. bolleyi* + *B. sorokiniana* tended to have a greater disease rating than those inoculated with the higher rate of *M. bolleyi* + *B. sorokiniana*. This was more obvious in July (Table 8.19), when plants inoculated with the low level of *M. bolleyi* + *B. sorokiniana* had a disease rating up to 45% greater than those inoculated with the high rate of *M. bolleyi* + *B. sorokiniana*. The corresponding differences in August (Table 8.20) and December (Table 8.21) were up to 27% and 21%, respectively.

TABLE 8.21: Disease rating (%) on subcrown internodes of Kite and Machete wheat at Caloote and Mannum in December, 1989. Values were calculated from a twenty plant sub-sample for each replicate. Inoculation treatments are described in Table 8.16. (Values are the mean of five replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	44.8	36.2	48.6	63.3
Mb2-F	44.9	54.0	51.4	57.1
Bs-F	45.6	37.9	43.9	70.6
Mb1Bs-F	36.9	45.7	54.7	67.6
Mb2Bs-F	59.2	63.7	56.5	53.2
F	42.2	49.8	57.6	53.8
Mean	45.6	47.9	52.1	60.9
Mb1-NF	50.4	44.5	50.8	55.3
Mb2-NF	42.5	45.4	49.9	46.5
Bs-NF	44.5	36.7	54.3	54.7
Mb1Bs-NF	48.4	51.6	50.2	41.6
Mb2Bs-NF	48.1	41.4	51.2	55.3
NF	50.5	52.7	56.6	50.5
Mean	47.4	45.4	52.2	50.7
LSD Treatments	17.2	17.1	15.6	14.2
LSD Means	8.7	11.9	6.0	10.3

Grain Yield

Overall, Machete had a slightly higher yield than Kite at both sites. At Caloote, Machete yielded 9 - 16% more than Kite, and 2 - 11% more at Mannum (Table 8.22). Fumigation actually reduced yields at Mannum, but at Caloote, yield of Kite and Machete,

respectively, was 21% and 14% higher on fumigated than on non-fumigated plots. These differences were significant (P<0.05) for both varieties at Caloote.

Inoculation with *B. sorokiniana* did not result in Machete (susceptible to *B. sorokiniana*) having a lower yield than Kite (moderately resistant). Some plots inoculated with *B. sorokiniana* had a yield up to 20% lower than those inoculated with *M. bolleyi*, but this difference was never significant (Table 8.22). Inoculation with *M. bolleyi* + *B. sorokiniana* sometimes resulted in a greater yield than inoculation with *B. sorokiniana* alone, but this was not statistically significant, and some plots inoculated with both fungi had a lower yield than those inoculated with only *B. sorokiniana*.

TABLE 8.22: Grain yield (g/two 1.2 m plot rows) of Kite and Machete wheat at Caloote and Mannum in December, 1989. Inoculation treatments are described in Table 8.16. (Values are the mean of five replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	122.0	128.3	139.8	135.8
Mb2-F	117.4	130.8	120.7	125.5
Bs-F	119.9	142.7	126.4	121.2
Mb1Bs-F	123.4	133.9	127.5	128.3
Mb2Bs-F	128.3	144.0	123.5	117.1
F	131.2	134.4	115.7	142.9
Mean	123.7	135.7	125.6	128.5
Mb1-NF	103.6	132.8	141.5	148.0
Mb2-NF	97.2	115.2	145.4	143.1
Bs-NF	104.8	115.2	116.0	158.3
Mb1Bs-NF	77.8	105.9	139.9	142.0
Mb2Bs-NF	98.5	130.5	133.8	170.3
NF	102.4	97.8	150.2	164.2
Mean	97.4	116.2	137.8	154.3
LSD Treatments	24.5	36.7	38.1	42.8
LSD Means	11.7	15.3	16.8	16.9

Protein Content of Grain

At Caloote, Kite produced grain with a higher protein content than that of Machete, but varieties were similar at Mannum (Table 8.23). Fumigation led to a higher protein content for both varieties at both sites. Overall, fumigation significantly (P<0.05)

enhanced protein content of both varieties at Caloote. Protein content of both varieties was, on average, 9% higher on fumigated than on non-fumigated plots.

Protein levels were not measured for inoculated treatments at Mannum. At Caloote, plots inoculated with *M. bolleyi* + *B. sorokiniana* usually produced grain with a higher protein content than that of plots inoculated with *B. sorokiniana* alone (Table 8.23), but this difference was not statistically significant. However, Machete on non-fumigated areas inoculated with *M. bolleyi* + *B. sorokiniana* had a lower grain protein content than that of those inoculated with *B. sorokiniana* alone.

TABLE 8.23: Protein content (%) of Kite and Machete wheat grain from Caloote and Mannum in December, 1989. Protein levels were determined at 11% moisture. Inoculation treatments are described in Table 8.16. (Values are the mean of three replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	13.0	11.5	...	...
Mb2-F	13.2	11.7	...	...
Bs-F	12.3	12.0	...	...
Mb1Bs-F	12.9	11.9	...	...
Mb2Bs-F	13.2	12.5	...	...
F	12.8	12.1	13.6	13.5
Mean	12.9	12.0	...	...
Mb1-NF	11.8	11.0	...	...
Mb2-NF	11.6	10.8	...	...
Bs-NF	11.5	11.3	...	...
Mb1Bs-NF	11.5	10.7	...	...
Mb2Bs-NF	12.3	10.7	...	...
NF	11.8	10.7	12.3	12.5
Mean	11.8	10.9	...	...
LSD Treatments	1.0	0.7	2.6	1.0
LSD Means	0.3	0.5	...	...

Weight of Crown Roots at Maturity

Overall, crown roots of Kite weighed up to 30% more than those of Machete (Table 8.24). Fumigation significantly ($P<0.05$) enhanced weight of Kite crown roots (by 35%) at Caloote, but the difference was not significant at Mannum. Machete crown root weight was increased by up to 25% due to fumigation, but this was not significant at

either site.

At Caloote, crown roots of Kite inoculated with *M. bolleyi* + *B. sorokiniana* on non-fumigated soil weighed up to 29% more than those inoculated with *B. sorokiniana* alone (Table 8.24). This difference was significant ($P<0.05$) for plants inoculated with the higher rate of *M. bolleyi* + *B. sorokiniana* compared with those inoculated with *B. sorokiniana* only. At Mannum, crown roots of both varieties on non-fumigated plots inoculated with *M. bolleyi* + *B. sorokiniana* weighed less than those inoculated with only *B. sorokiniana.*

TABLE 8.24: Dry weight of crown roots (g/plant) on Kite and Machete wheat plants at Caloote and Mannum in December, 1989. Values were measured on a twenty plant sub-sample from each replicate. Inoculation treatments are described in Table 8.16. (Values are the mean of five replicates for each treatment).

	CALOOTE		MANNUM	
Treatment	**Kite**	**Machete**	**Kite**	**Machete**
Mb1-F	2.0	1.1	2.2	1.6
Mb2-F	2.5	1.6	2.5	1.8
Bs-F	2.0	1.6	2.4	2.1
Mb1Bs-F	2.6	1.5	3.4	2.1
Mb2Bs-F	2.5	1.8	3.5	2.4
F	2.2	2.1	2.2	1.4
Mean	2.3	1.6	2.7	1.9
Mb1-NF	1.3	1.1	1.5	1.1
Mb2-NF	1.5	1.1	2.0	1.4
Bs-NF	1.1	1.0	2.8	2.0
Mb1Bs-NF	1.2	1.6	2.0	1.7
Mb2Bs-NF	2.0	1.2	2.1	1.7
NF	1.6	0.9	2.2	1.7
Mean	1.5	1.2	2.1	1.6
LSD Treatments	0.8	0.7	1.4	0.8
LSD Means	0.3	0.5	0.8	0.4

Isolation of Microdochium bolleyi and Bipolaris sorokiniana

Graphs of percentage isolation frequencies are presented (Figures 8.8 - 8.15), but statistical analyses were performed on transformed data. The corresponding transformed data are in Appendix B.

All isolation frequencies were low in July (Figures 8.8 - 8.11), but had increased

by August (Figures 8.12 - 8.15). *M. bolleyi* was always more common than *B. sorokiniana*, even on the subcrown internodes, and especially on the non-fumigated areas. Isolation frequencies of *B. sorokiniana* were extremely low, and *B. sorokiniana* was often not isolated from many treatments, including some that were inoculated with this species.

Inoculated plots often had higher infection levels than the corresponding uninoculated plots. This was true for both species, but rarely significant. Inoculation of plots with *M. bolleyi* was more successful than inoculation with *B. sorokiniana*. Overall, inoculation with *M. bolleyi* increased infection levels from 6% to 8%, and *B. sorokiniana* infection only increased from 2% to 2.5%. *M. bolleyi* inoculum was as efficient on fumigated as on non-fumigated plots, while *B. sorokiniana* inoculum was more successful on the non-fumigated areas. Inoculation significantly enhanced infection levels in two instances only. In August, seminal roots from non-fumigated plots of Kite inoculated with *M. bolleyi* at Mannum were infected with significantly ($P<0.05$) more *M. bolleyi* than those from the uninoculated plots (Figure 8.13). In this case, plants from inoculated plots were infected with *M. bolleyi*, but those from uninoculated plots were not. Similarly, subcrown internodes of Machete from inoculated plots at Mannum were infected with 68% more *B. sorokiniana* than were uninoculated plots in August (Figure 8.14), and this was significant ($P<0.05$). Both species were also isolated from fumigated plots that were not inoculated, although non-fumigated plots were usually infected to a higher degree than the fumigated plots.

In some cases, plants inoculated with the high rate of *M. bolleyi* were infected with more *M. bolleyi* than those inoculated with the low level of this species. However, these differences were never significant.

Inoculation with *M. bolleyi* + *B. sorokiniana* had little effect on the frequency of *B. sorokiniana* isolated. The difference in *B. sorokiniana* frequency between *M. bolleyi* + *B. sorokiniana* and *B. sorokiniana* treatments was rarely significant.

FIGURE 8.8: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of Machete wheat at Caloote and Mannum in July, 1989. (Values are the mean of four replicates for each treatment). Plots on areas treated with methyl bromide soil fumigant (F) or on adjacent untreated areas (NF) were inoculated as follows: *M. bolleyi* was applied at low (Mb1) and high (Mb2) rates, alone and in conjunction with *B. sorokiniana* (Mb1Bs and Mb2Bs); *B. sorokiniana* was applied at one inoculum level only (Bs). Control plots (F and NF) were inoculated with neither fungus.

FIGURE 8.9: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of Kite wheat at Caloote and Mannum in July, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of four replicates for each treatment).

FIGURE 8.10: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from subcrown internodes of Machete wheat at Caloote and Mannum in July, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of four replicates for each treatment).

Figure 8.8

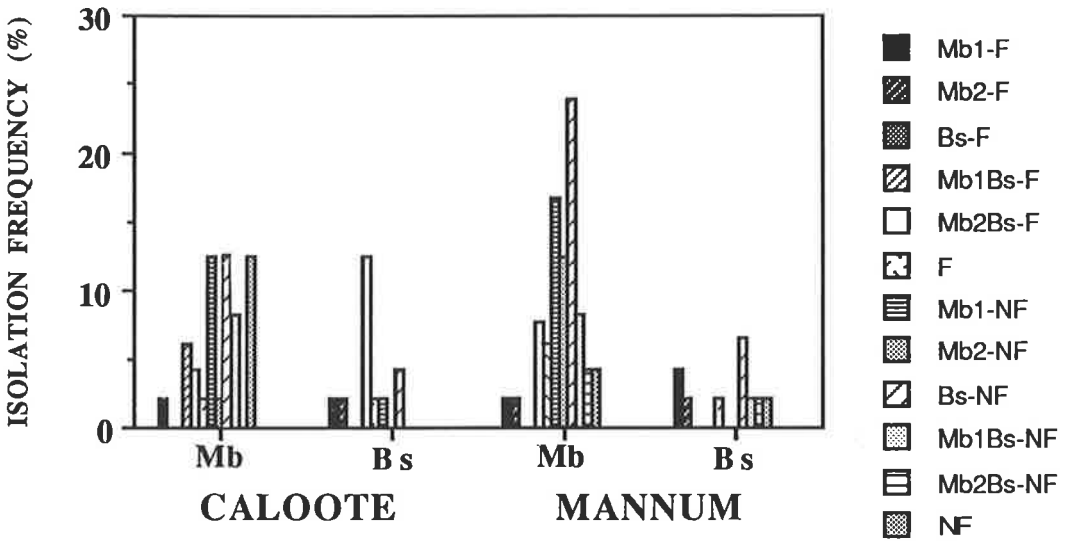


Figure 8.9

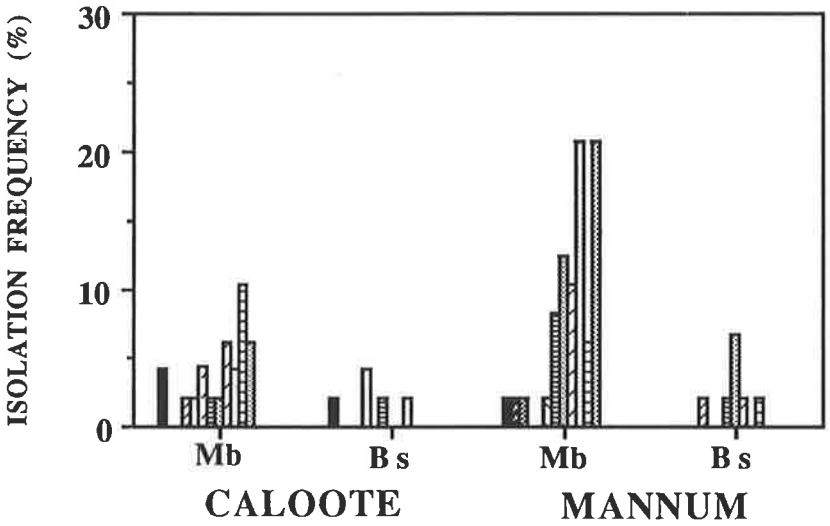


Figure 8.10

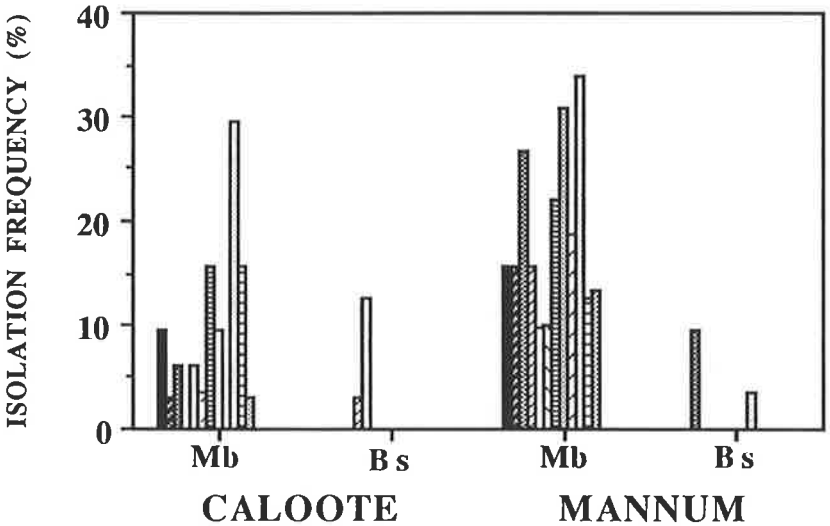


FIGURE 8.11: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorckiniana* (Bs) isolated from subcrown internodes of Kite wheat at Caloote and Mannum in July, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of four replicates for each treatment).

FIGURE 8.12: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of Machete wheat at Caloote and Mannum in August, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of three replicates for each treatment).

FIGURE 8.13: Frequency (% root segments infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from seminal roots of Kite wheat at Caloote and Mannum in August, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of three replicates for each treatment).

Figure 8.11

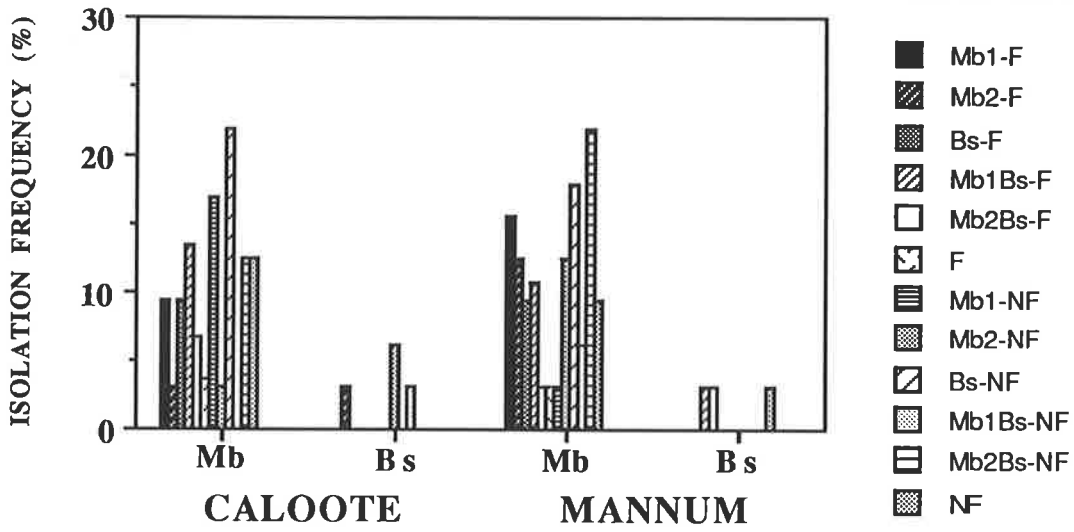


Figure 8.12

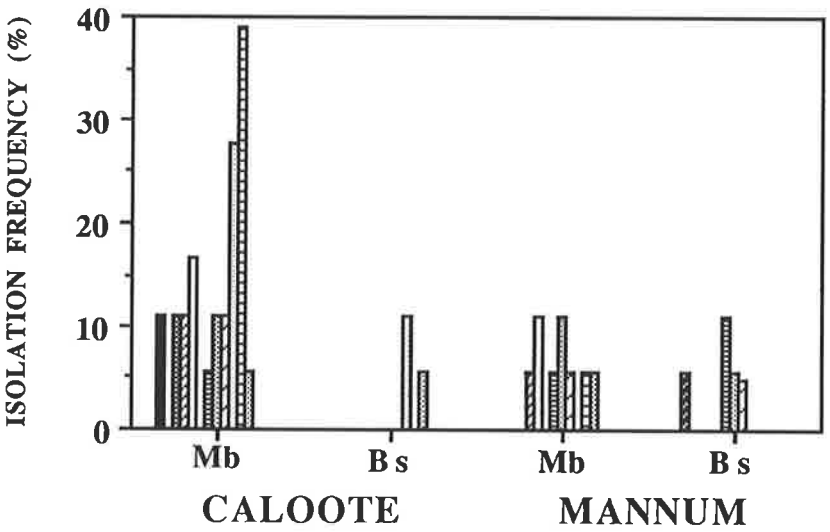


Figure 8.13

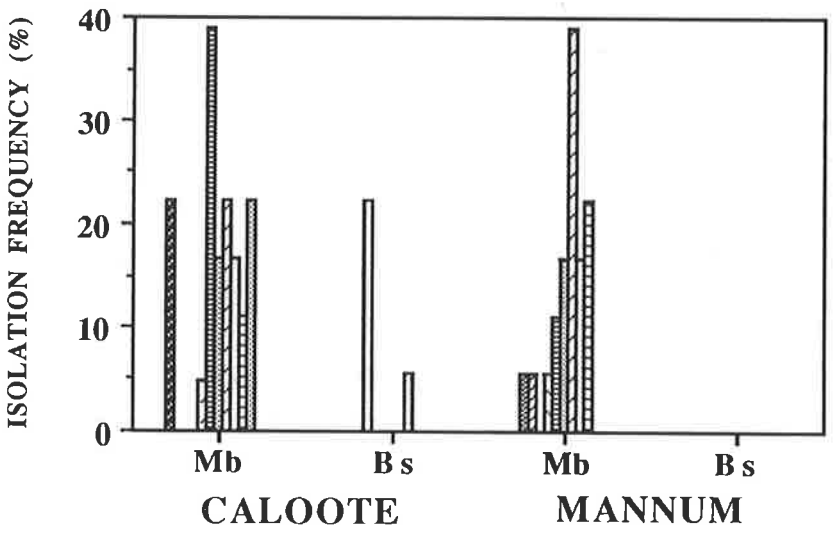


FIGURE 8.14: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from subcrown internodes of Machete wheat at Caloote and Mannum in August, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of three replicates for each treatment).

FIGURE 8.15: Frequency (% subcrown internodes infected) of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) isolated from subcrown internodes of Kite wheat at Caloote and Mannum in August, 1989. Inoculation treatments are described in Figure 8.8. (Values are the mean of three replicates for each treatment).

Figure 8.14

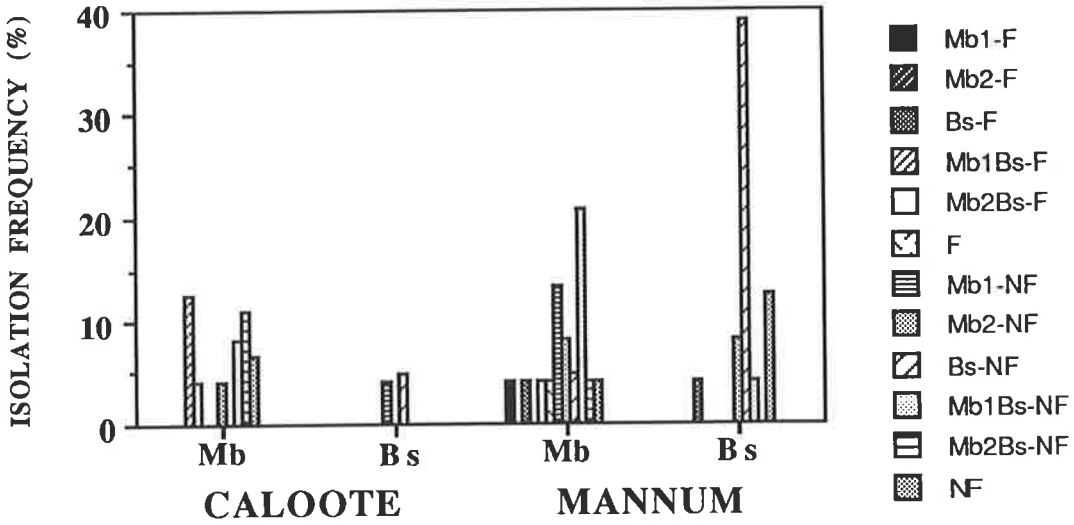
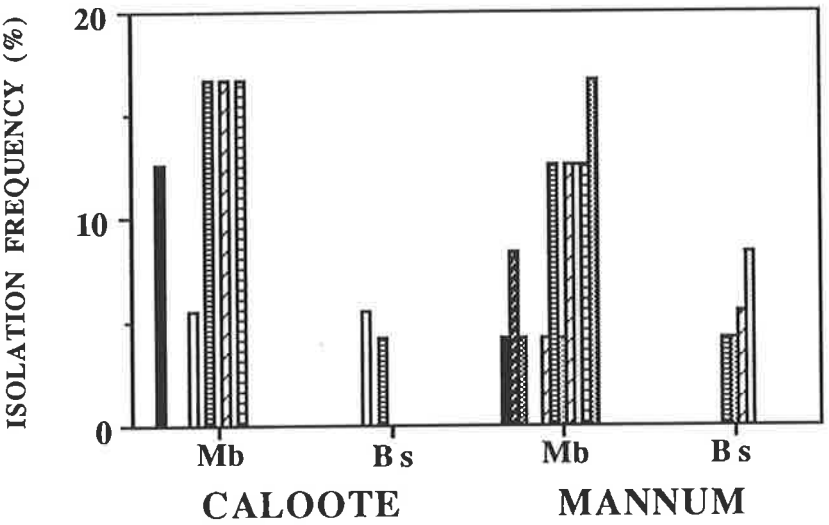


Figure 8.15



8.3.3.3 Summary of Results

Inoculation in the field provided less conclusive evidence that *M. bolleyi* reduces the extent of damage caused by *B. sorokiniana* than did experiments under controlled conditions in pots or in Petri plates. Despite the large number of replicates planted in the field, the variation between replicates was high, and differences between values were rarely statistically significant. It appears that the effects of inoculation were often obscured by climatic and edaphic factors, and especially by the presence of many other organisms in the soil. However, the general trend in 1988 and 1989 did follow that which was observed in pots or in Petri plates, although differences between treatments were not always significant.

(1) Fumigation enhanced plant growth in both 1988 and 1989. Shoot weight was greater on fumigated than on non-fumigated plots (Tables 8.13, 8.16 - 8.18), and fumigation increased crown root mass of most treatments in 1989 (Table 8.24).

(2) In 1988, up to 34% of seedlings died before maturity, and fumigation partially alleviated the incidence of seedling death at one of the sites studied (Table 8.15).

(3) Fumigation increased grain yield at Mannum in 1988 by 40%, but yield at Sanderston was not enhanced by fumigation (Table 8.15). Conversely, in 1989, yield at Mannum was not increased by fumigation, but was 14 - 21% higher at Caloote due to this treatment (Table 8.22). Protein content of grain, measured in 1989, was increased by fumigation (Table 8.23).

(4) Machete (susceptible to *B. sorokiniana*) and Kite (moderately resistant) were compared in 1989. However, results were not consistent with the varietal reactions to *B. sorokiniana*. Interaction between *M. bolleyi* and *B. sorokiniana* did not seem to be influenced by the varietal reaction to *B. sorokiniana.*

(5) The number of plants per plot row in November was affected little by inoculation (Table 8.15).

(6) Inoculation had no significant effect on grain yield (Tables 8.15, 8.22).

(7) Inoculation with *M. bolleyi* enhanced infection levels, although inoculation with *B. sorokiniana* was not necessarily as successful (Figures 8.1 - 8.15). *B. sorokiniana*

achieved much lower infection levels than did *M. bolleyi*, even on the subcrown internodes.

(8) The high rate of *M. bolleyi* inoculum used in 1989 did not appear to reduce damage caused by *B. sorokiniana* to a greater extent than did the low inoculum level used in conjunction with *B. sorokiniana* (Figures 8.8 - 8.15).

8.4 DISCUSSION

Microdochium bolleyi did not augment root disease caused by *Bipolaris sorokiniana*, nor were the symptoms observed under field conditions reproduced by inoculation treatments combining these two fungal pathogens. Plants inoculated with *M. bolleyi* + *B. sorokiniana*, at least under controlled conditions, actually tended to suffer less damage than those inoculated with *B. sorokiniana* alone, disproving the original hypothesis that these species combined may produce more severe root disease. Neither *M. bolleyi* nor *B. sorokiniana*, alone or in conjunction, is responsible for the root rotting condition seen on the lighter soil types of the Murray Mallee and elsewhere in the South Australian wheat belt. This suggests that another organism, or combination of organisms, is responsible for this root damage, or that the conditions required to reproduce the symptoms were not present in pot experiments.

Competition between the two species was seen on plants in pots (Tables 8.1 - 8.11) or seedlings in Petri plates (Table 8.12), but was not clearly demonstrated under field conditions (Tables 8.13 - 8.24, Figures 8.1 - 8.15). Such an interaction under controlled conditions, although significant, is no guarantee that the two organisms will behave similarly under field conditions. The differences in growing conditions, water and nutrient status for the three types of experiment make comparisons between the results difficult. Due to considerable variation within treatments and between sample dates in the field, and because effects of the different inoculation treatments were inconsistent, the results must be interpreted with caution. Climatic, edaphic and biotic factors are no doubt largely responsible for this variation. Until these sources of variation are defined and controlled, the exact role played by these organisms in the field, and the interaction that

occurs between *M. bolleyi* and *B. sorokiniana*, cannot be fully ascertained.

Others have encountered similar problems with such experiments. Cotterill and Sivasithamparam (1988, 1989) found that results were inconsistent and rarely significant when they inoculated field plots with *Gaeumannomyces graminis*. Plants grown in the field were subject to infection by many species of fungi, even on areas treated with methyl bromide soil fumigant. Soil is an intensely competitive environment for pathogens, and introduced control agents are also vulnerable to antagonism from other soil-borne organisms (Deacon, 1988). Such factors also acted to obscure the results from field experiments reported here. Inoculation with *M. bolleyi* did, however, lead to enhanced levels of infection in the field. Inoculation with *B. sorokiniana* was not as successful, although infection levels did tend to increase later in the growing season.

The effect of *B. sorokiniana* on the growth and disease level of wheat plants was not consistent with the known varietal reactions of cultivars to this pathogen. Such an observation is explicable under field conditions, where numerous other organisms infect roots, with climatic and edaphic factors also obscuring the varietal response to *B. sorokiniana*. However, inconsistent varietal reactions were also observed under controlled conditions in pots and in Petri plates, and this cannot be readily explained, unless the *B. sorokiniana* isolate used differed somehow from those usually investigated. In pot Experiment 2 (Section 8.1.3.2), Kite (moderately resistant) subcrown internodes were infected more by *B. sorokiniana* than were those of the moderately susceptible variety Condor (Table 8.6), but the reverse was true in Experiment 3 (Table 8.9). *B. sorokiniana* reduced plant weight of Kite, but had little deleterious effect on the weight of Condor plants (Tables 8.7, 8.10). Kite, in Experiment 2 (Table 8.8), had a higher disease rating than Condor, but both varieties had similar disease rating values in Experiment 3 (Table 8.11). Inoculating seedlings in Petri plates (Table 8.12) with *B. sorokiniana* reduced leaf number on Kite more so than on Machete (by 34% and 21%, respectively), even though Kite is moderately resistant to *B. sorokiniana* and Machete susceptible. The same was true for root length (reduced by 30% for Kite; no reduction on Machete), shoot length (reduced by 28% for Kite and 16% for Machete) and plant weight (reduced by 16% for Kite and 10% for Machete). Kite coleoptiles were lesioned to a greater extent

than those of Machete inoculated with *B. sorokiniana.*

In South Australia, P. J. L. Whittle (personal communication) recorded a yield loss of 16% in Machete (susceptible), but *B. sorokiniana* did not adversely affect the yield of moderately resistant varieties. However, in Queensland, Wildermuth (1985) found that the yield of Kite (moderately resistant) could still be reduced by up to 19%. This suggests that varietal response to *B. sorokiniana* depends on growing conditions and inoculum levels in the soil. Furthermore, varieties may be tolerant rather than resistant, and resistance to *B. sorokiniana* is incomplete, hence the designation "moderately" resistant.

Varietal reaction to *B. sorokiniana* had little effect on the apparent antagonism between *M. bolleyi* and *B. sorokiniana.* When inoculum was placed below the seed in pots (Section 8.1.3.2), *B. sorokiniana* infection of Kite was reduced 50 - 73% by inoculation with both fungi, and by 54% on Condor (Table 8.6). The reverse was true when inoculum was placed both above and below the seed (Section 8.1.3.3): inoculation with both species reduced *B. sorokiniana* infection on Kite by 29 - 55% and that on Condor by 56 - 70% (Table 8.9). In plates, inoculation with *M. bolleyi* + *B. sorokiniana* reduced the deleterious effect of *B. sorokiniana* on leaf number and root length of Kite more than it reduced the effect of *B. sorokiniana* on the growth of Machete (Table 8.12). The reverse was true, however, for shoot length. *M. bolleyi* + *B. sorokiniana* reduced lesioning of Kite coleoptiles due to *B. sorokiniana* by 73% and reduced lesioning of Machete coleoptiles by 40%.

Besides the interaction between *M. bolleyi* and *B. sorokiniana,* another important result was obtained from field experiments. At field sites in 1988, up to 34% of seedlings died before the crop reached maturity (Table 8.15). Fumigation alleviated the incidence of seedling death to some extent, but soil-borne organisms were not solely responsible for the discrepancy between seedling number in late June-early July and plant number recorded at harvest in November. The time of seedling death was not ascertained, nor was the distribution of dead plants determined. However, it appears that plant death occurred along rows at random, as patches containing dead plants or areas devoid of plants were not seen. Seedling death was not obvious until plant numbers recorded at

emergence and at maturity were compared at the end of the growing season. The measured loss of plants represents post-emergent death, but some seedlings would have died prior to emergence. *B. sorokiniana* can cause seedling blight under some conditions, as can *Rhizoctonia solani* and *Pythium* spp. (Wiese, 1987). It is, however, doubtful that *B. sorokiniana*, at the infection levels recorded at Murray Mallee field sites, caused pre- or post-emergent seedling death. *Pythium* was recorded at high levels (Chapters 5 and 9) and may have killed seedlings, but *R. solani* was isolated infrequently (Chapters 9 and 10). A loss of plants between seeding and maturity is often observed in commercial crops in these regions, but no explanation has been forthcoming.

M. bolleyi and *B. sorokiniana* both colonised the subcrown internodes of wheat plants. Disease rating, measured as the severity and occurrence of subcrown internode lesioning, was reduced in the presence of *M. bolleyi*. This was also reflected in the reduction in *B. sorokiniana* frequency in isolations from the subcrown internodes of plants inoculated with both species of fungi. *M. bolleyi* caused no visible damage to subcrown internodes in pots (Section 8.1.3), resulting in a disease rating of 0%. *M. bolleyi* was, however, isolated from subcrown internodes in pot experiments, often at frequencies as high as that of *B. sorokiniana*. Scardaci and Webster (1982), on the other hand, did observe necrosis of subcrown internodes on plants inoculated with *M. bolleyi*. Inoculation with *M. bolleyi* caused lesioning of coleoptile tissues on seedlings in Petri plates (Section 8.2.3), although this damage was much less than that caused by inoculation with *B. sorokiniana*, and *M. bolleyi* reduced the damage caused by *B. sorokiniana* when seedlings were inoculated with both species of fungi.

The mechanism whereby *M. bolleyi* reduces the damage caused by *B. sorokiniana* seems to be one of competition. *M. bolleyi* colonises tissues rapidly, achieving high levels of infection, without causing severe damage to plants. *B. sorokiniana* is thus out-competed for colonisation of host tissues. Plants infected by *M. bolleyi* are not readily invaded by *B. sorokiniana*, and thus sustain little damage. *M. bolleyi* seems to inhibit *B. sorokiniana*, although there was no visible antagonism between the two species in culture (Section 8.1.3). *M. bolleyi* seems to compete with *B. sorokiniana, in vivo*, for host substrate. The role of competition is further supported by the observation that infection

with *B. sorokiniana* also reduces colonisation of tissues by *M. bolleyi* to some extent.

This is supported by the results of other workers. Reinecke (1978) saw no symptoms on wheat inoculated with *M. bolleyi*, but readily re-isolated the fungus from almost every plant. *M. bolleyi* caused limited damage, despite heavy colonisation of plant tissues (Murray and Gadd, 1981), and had no significant effect on yield (Reinecke, 1978; Kane *et al.*, 1987). Kruger (1976) concluded that *M. bolleyi* is non-pathogenic or only weakly so, and is less pathogenic to cereals than other fungi that cause root rot (Scardaci and Webster, 1982; Sturz and Bernier, 1987a, 1987b). Although *M. bolleyi* is frequently isolated from cereals in the field and makes up a large proportion of the fungal community (Sharp, 1959; Hoes, 1962, 1966; Waller, 1968; Salt, 1977; Reinecke, 1978; Reinecke and Fokkema, 1981; Scardaci and Webster, 1982; Sturz and Bernier, 1985, 1987b, 1989; Kane *et al.*, 1987), it is often as common on healthy plants as it is on diseased plants (Hoes, 1962, 1966; Waller, 1968, 1979; Murray, 1981; Murray and Gadd, 1981).

Pathogens are generally excluded from a root region already colonised by biocontrol agents, and the success of a control agent depends on its ability to gain prior occupancy of the infection court, and that populations of the antagonist exceed those of the pathogen (Deacon, 1988). *M. bolleyi* is capable of fulfilling these criteria. Fungi that produce the highest number of propagules or the greatest mass of mycelium have the greatest competitive advantage (Faull, 1988). High competitive saprophytic ability depends on several factors that allow fungi to exploit favourable habitats and survive in the soil: rapid spore germination, high growth rate, high concentration of extracellular enzymes, the production of and tolerance to antibiotics, profuse sporulation, and the production of thick-walled bodies that survive in the soil under adverse conditions (Faull, 1988). *M. bolleyi* possesses most of these attributes, although production of antibiotics by this species has not been demonstrated. Thick-walled chlamydospores are formed by *M. bolleyi*, whereas *B. sorokiniana* survives in the soil as conidiospores. *M. bolleyi* also shows high enzymatic activity in the breakdown of pectin, xylan and carboxymethyl-cellulose (Domsch and Gams, 1969).

B. sorokiniana is considered to have a low competitive saprophytic ability (Harding, 1973), whereas *M. bolleyi* seems to be a much more successful competitor in

the host-soil environment. However, Harding (1973) found little evidence of fungi antagonistic to *B. sorokiniana* in Canadian wheat samples, where this species frequently infects up to 80% of subcrown internodes (Sallans and Tinline, 1965; Harding, 1973). *B. sorokiniana* infects a smaller proportion of subcrown internodes under Australian conditions (Purss, 1970), where it usually causes less damage to wheat crops than in Canada. In fact, Tinline (1984) reported that only 10 - 22% of subcrown internodes sampled in South Australia were infected by *B. sorokiniana*. Harding (1973) concluded that the ability of *B. sorokiniana* to invade such a large proportion of available subcrown internode tissue, and the apparent absence of antagonists, compensates for its low competitive saprophytic ability. In 1988 and 1989, up to 10 - 12% of subcrown internodes from untreated plots were infected with *B. sorokiniana*, but as many as 16 - 21% were colonised by *M. bolleyi*. The situation in South Australia is somewhat different from that in Canada, with *M. bolleyi* and *Fusarium* spp. (Chapter 4) infecting more subcrown internodes than does *B. sorokiniana*.

The role of natural senescence of root cortices is thought to be important in interactions involving *M. bolleyi*. Cortical senescence may allow weakly pathogenic fungi, like *M. bolleyi*, to invade root cortices as cell resistance declines. The cortical cells of cereal roots senesce naturally, even in the absence of pathogens or other microorganisms, and long before roots show any evidence of cortical browning or sloughing (Holden, 1975, 1976). Under glasshouse conditions, it is not unusual for several cortical cell layers to be incapable of defence in only six to ten day old regions of wheat roots. *M. bolleyi* can thus colonise senescing cortical tissues with no detrimental effect to the host plant (Kirk and Deacon, 1987a, 1987b). *M. bolleyi* forms dormant chlamydospores within root cells, and this is thought to be due to the host plant's response to infection, because the fungus cannot continue to grow in living root cells (Murray 1981; Murray and Gadd, 1981). Chlamydospores are sometimes seen within root cells of plants that have been inoculated with *M. bolleyi* (Chapter 7), but the fungus is also readily isolated from root segments not containing these spores. This suggests that *M. bolleyi* is quite capable of growing in living root cells. Chlamydospores are rarely seen in field samples, but *M. bolleyi* can be isolated from roots and subcrown internodes

at relatively high frequencies. The role of cortical senescence in the interaction between *M. bolleyi* and *B. sorokiniana* (under the conditions of experiments reported here) is thus doubtful.

Cortical senescence may, however, play an important part in some interactions between fungi. *Phialophora graminicola* is a non-pathogenic parasite that seems to depend on cortical senescence for infection of roots (Holden, 1976; Deacon, 1980; Deacon and Lewis, 1986). This fungus can restrict the occurrence of *Gaeumannomyces graminis* var. *tritici* in cereal crops (Deacon, 1973; Slope *et al.*, 1978). *G. graminis* var. *graminis* and a *Phialophora* sp. have also been shown to control *G. graminis* var. *tritici* under field conditions in Australia (Wong and Southwell, 1980). Unlike *B. sorokiniana*, *G. graminis* var. *tritici* infects senescing cortical tissues, and these control agents compete with *G. graminis* var. *tritici* by excluding it from the senescing root areas (Deacon and Henry, 1980; Kirk, 1984).

In the glasshouse, *M. bolleyi* controls *G. graminis* var. *tritici* as effectively as does *P. graminicola* (Kirk and Deacon, 1987a, 1987b). Like *P. graminicola*, *M. bolleyi* is thought to compete with *G. graminis* var. *tritici* for colonisation of senescing cortical tissues, progressively decreasing the number of roots infected by *G. graminis* var. *tritici* as *M. bolleyi* inoculum levels are increased (Kirk and Deacon, 1987a). Kirk and Deacon (1987a) did not test the efficacy of *M. bolleyi* in controlling *G. graminis* var. *tritici* in the field, but as the results of their tests were similar to those in which *G. graminis* var. *tritici* is controlled by other weakly or non-pathogenic fungi, they assume that significant control of *G. graminis* var. *tritici* would be attainable under field conditions.

It has been suggested that *P. graminicola* controls *G. graminis* var. *tritici* on cereals due to induced host resistance (Speakman and Lewis, 1978). This is, however, unlikely because *G. graminis* var. *tritici* sometimes reduces colonisation of roots by control agents (Deacon, 1974a, 1974b, 1981). A similar situation exists in the interaction between *M. bolleyi* and *B. sorokiniana*: *M. bolleyi* reduces the incidence of and damage caused by *B. sorokiniana*, and *B. sorokiniana* (to some extent) reduces the frequency of *M. bolleyi* in host tissues (Section 8.1.3). This further supports the role of competition in the protection from *B. sorokiniana* afforded to plants by *M. bolleyi*.

M. bolleyi is not antagonistic to *G. graminis* var. *tritici* in culture (Kirk and Deacon, 1987a), nor was it antagonistic to *B. sorokiniana* in the absence of a host plant. This suggests that *M. bolleyi* is not capable of producing effective quantities of antibiotics, if any, and it is not mycoparasitic. Antagonism to root pathogens only occurs *in vivo*, by competition for the possession of host substrate.

Weak parasites can contribute to disease complexes, but others are able to control "major" root pathogens (Deacon, 1988). *M. bolleyi* is frequently associated with cereals suffering root rot in North America (Sharp, 1959; Hoes, 1962, 1966; Scardaci and Webster, 1982; Sturz and Bernier, 1985, 1987a; Kane *et al.*, 1987) and in South Australia (Harris, 1986). Kane *et al.* (1987) proposed that *M. bolleyi* was a potentially important component of the root disease complex. Although *M. bolleyi* apparently protects plants from some root pathogens and usually causes negligible damage to cereals, it can become damaging under certain conditions. *M. bolleyi* is pathogenic to cereals stressed by low temperature (Sturz and Bernier, 1987a) or by water-logging (Black and Brown, 1986), and wheat crops grown on light, sandy soils in the Netherlands suffer yield reductions due to infection with *M. bolleyi* (Tichelaar, 1978). However, antagonism between the components of the root disease complex is possible. *Fusarium culmorum* inhibits the growth of *B. sorokiniana* in culture, where it interferes with spore germination (Ledingham, 1942). *In vivo*, Scardaci and Webster (1981) detected antagonism between *F. graminearum* and *B. sorokiniana* on barley plants, resulting in less seedling blight and root rot. The pathogen introduced first was isolated most frequently, as prior colonisation and substrate possession tend to exclude other organisms. A similar situation is likely to occur with *M. bolleyi* and *B. sorokiniana* on wheat.

Competition between organisms has little or no effect on the viability of pathogens (Baker, 1981). However, *M. bolleyi* is able to compete with *B. sorokiniana* for infection of host tissues, and thus has a greater opportunity for reproduction. This would then reduce the number of *B. sorokiniana* propagules available to subsequently infect plants. *B. sorokiniana* survives in the soil as conidia, and as a saprophyte on crop debris. Some samples of root and crown fragments were examined prior to seeding in 1989, when *B.*

sorokiniana colonised less than 2% of crop debris fragments, while *M. bolleyi* infected up to 7% of the debris tested. *M. bolleyi* has a greater competitive saprophytic ability than *B. sorokiniana*, therefore excluding it from both living and dead host tissues. In the long term, high levels of *M. bolleyi* infection would reduce the number of available propagules of *B. sorokiniana* in the soil, thus reducing the pathogen's potential for crop damage.

M. bolleyi occurs frequently on wheat plants in South Australia, at least in the Murray Mallee where field experiments were conducted. Limited sampling in other areas, however, suggests that *M. bolleyi* is a frequent coloniser of wheat roots in most cereal-growing areas of the State. Harris (1986) and Harris and Moen (1985a, 1985b) also found that *M. bolleyi* was a common inhabitant of cereal roots in South Australia.

The incidence of *P. graminicola*, introduced to control *G. graminis* var. *tritici*, declines in cereal monoculture, whereas that of *M. bolleyi* does not (Deacon, 1988). In fact, *M. bolleyi* increases in frequency under successive cereal crops, especially wheat. In Manitoba, the incidence of *M. bolleyi* was high under continuous wheat crops (Sturz and Bernier, 1989), increasing in incidence with each successive crop (Sturz and Bernier, 1987b). Domsch and Gams (1968) and Domsch *et al.* (1968) also reported that *M. bolleyi* was common in soils repeatedly cropped to wheat, and Sturz and Bernier (1985) found that the frequency of *M. bolleyi* was higher on wheat grown after wheat than after other crops, including barley and oats. Once established within a niche, the disease antagonist can prevent ingress of the pathogen for as long as that niche exists (Faull, 1988). *M. bolleyi* already occurs in agricultural soils, so has an established niche in the host-soil environment.

"Minor" pathogens, like *M. bolleyi*, play an important role in displacing major pathogens in cereal root and crown tissues (Wong, 1985). However, control of one disease can create a biological "vacuum", enabling others to assume greater importance (Kreutzer, 1965). This phenomenon is unlikely to occur in situations where a pathogen is excluded from plant tissues due to colonisation by a non-pathogenic or weakly pathogenic species like *M. bolleyi*. Presumably, exclusion of one pathogen due to competition brought about by prior substrate colonisation and high infection levels, would also exclude other pathogens. Cases exist where a control agent is specifically antagonistic to

certain pathogens, but this is more likely to occur in systems involving mycoparasitism or the production of antibiotic substances.

M. bolleyi seems non-specific in its control of root pathogens, and has been reported to control several species of fungi in cereal roots, at least on an experimental basis. Control of *G. graminis* var. *tritici* has already been discussed, and *M. bolleyi* is also antagonistic to *Pythium* spp. (Salt, 1979). Disease caused by *Pseudocercosporella herpotrichoides* (the causal agent of cereal eyespot) can be reduced by up to 80% in the presence of *M. bolleyi*, and the amount of *Fusarium* spp. isolated from wheat and rye decreases when plants are inoculated with *M. bolleyi* (Reinecke, 1978).

Waller (1979) inoculated wheat and barley with *M. bolleyi* plus *Pythium arrhenomanes* to determine whether *Pythium* infection was augmented in the presence of *M. bolleyi*. It was not, but Waller (1979) failed to notice that *M. bolleyi* in fact reduced the level of *Pythium* infection. The percent of diseased wheat roots was reduced from 23% to 12% when both fungi were present. Barley roots suffered 45% infection with *P. arrhenomanes* alone, but only 10% infection when *M. bolleyi* was also inoculated onto plants. Plant height was also nearer the control value when wheat and barley were inoculated with both species of fungi.

By virtue of its habitat, *B. sorokiniana* is subject to antagonism from various members of the soil flora. Numerous bacteria, actinomycetes and fungi inhibit *B. sorokiniana* and reduce the severity of disease (Henry, 1931; Anwar, 1949; Sallans, 1965; Old, 1965). Species of *Trichoderma* and *Gliocladium* are widely distributed in the soil (Domsch *et al.*, 1980), and produce antibiotics and other toxic metabolites that inhibit pathogenic fungi (Weindling and Emersen, 1936; Weindling, 1941; Tyner, 1966; Howell and Stipanovic, 1983; Papavizas, 1985). *G. roseum* and *T. viride* markedly reduced the pathogenicity of *B. sorokiniana* to barley and inhibited the germination of spores (Tyner and McKinnon, 1964). Plants inoculated with *B. sorokiniana* were subjected to 52% infection after three weeks, whereas inoculation with *B. sorokiniana* plus *T. viride* or *G. roseum* resulted in only 10% and 2% infection, respectively. Unlike *M. bolleyi*, *T. viride* causes breakdown and distortion of *B. sorokiniana* spores in culture, and strongly inhibits their pathogenicity (Campbell, 1956). *T. lignorum* also suppresses disease

caused by *B. sorokiniana* on wheat (Bisby *et al.*, 1933) and barley (Anwar, 1949).

Some years ago, Henry (1931) recognised that *B. sorokiniana* produced less disease on plants growing in unsterile than in sterile soil, due to competition and inhibition by other organisms in the soil. Many species of fungi have since been found to inhibit *B. sorokiniana* and reduce its pathogenicity. *Actinomucor repens*, *Sclerotinia trifoliorum* and *Myrothecium verrucaria* are slightly inhibitory, while *Phoma humicola* and *Epicoccum purpurascens* greatly reduce the pathogenic activities of *B. sorokiniana* (Campbell, 1956). Greaney and Machacek (1935) reported that *Cephalothecium roseum* reduced the pathogenicity of *B. sorokiniana* to plants in pots. Anwar (1949) found that root rot of barley was less severe when a *Penicillium* sp. was present. It is possible that symptomless invasion of plants by *Penicillium* spp., for example, interferes with subsequent invasion by *B. sorokiniana* (Chinn, 1971). A similar hypothesis probably applies to *M. bolleyi*, although *Penicillium* spp. produce antibiotics, whereas it is unlikely that *M. bolleyi* produces any metabolites toxic to other organisms.

Reinecke (1978) noted that the antagonistic capacity of *M. bolleyi* seemed remarkable. This fungus possesses the properties of a potentially successful biocontrol agent, as outlined by Deacon (1988), and the features affording an organism a high competitive saprophytic ability (Faull, 1988). Deacon (1988) considers that *M. bolleyi* has the attributes of a potentially commercial biocontrol agent, although it has not previously been tested in the field. In the work reported here, *M. bolleyi* was less successful as an antagonist to *B. sorokiniana* under field conditions (Section 8.3) than in the controlled environment of the laboratory (Section 8.2) or glasshouse (Section 8.1). Many opportunities exist for competition between organisms in the soil, and perhaps *M. bolleyi* is also subjected to competition from the plethora of organisms inhabiting agricultural soils. However, with better methods of administering inoculum to the soil, and at higher inoculum levels, *M. bolleyi* may fulfil the expectations of Reinecke (1978) and Deacon (1988) as a biocontrol agent.

Although *B. sorokiniana* is considered to be an important pathogen of wheat in South Australia, *M. bolleyi* achieved much higher infection levels than *B. sorokiniana* in the field, even on the subcrown internodes. The naturally high levels of *M. bolleyi* in the

soil may thus be a mitigating factor on disease caused by *B. sorokiniana* in this State.

CHAPTER 9

FUNGI ASSOCIATED WITH DETERIORATION OF WHEAT CROWN ROOTS

9.1 INTRODUCTION

The progressive deterioration of crown roots, and the associated inhibition of lateral root growth, was followed throughout the 1989 growing season. Extensive damage to crown root systems is commonplace in South Australian cereal crops, especially on the lighter soil types. Lateral roots along crown root axes consequently grow poorly, and long sections of root are devoid of laterals and root hairs, as described in Chapter 6. Organisms associated with this root damage were identified on Machete wheat at the Mannum and Caloote field sites (Figure 3.1).

Some of the symptoms observed were consistent with those that have been ascribed to *Rhizoctonia solani*: reddish-brown root tips tapering to a "spear" point, decortication, roots shortened and stiffened (Hynes, 1937a; Wiese, 1987). However, many species of fungi (along with the root lesion nematode, *Pratylenchus neglectus*) were identified in rotted crown roots. The role of *R. solani* in causing the observed damage to crown roots, under the circumstances investigated, is unlikely for reasons that will be described and discussed.

9.2 METHODS

Machete wheat was sampled from the uninoculated border plots (fumigated and non-fumigated) included in field experiments (sown on June 1, 1989) on the properties of B. Ramm (Mannum) and D. Abraham (Caloote). The design of these experiments is described in Chapter 8 (Sections 8.3.1 and 8.3.2), and all methods employed in field experiments are outlined in the General Methods.

Isolation of Fungi

Plants were sampled from both fumigated and non-fumigated plots seven times, approximately one week apart, from late July to mid-September. At each sample date, five plants were removed from each of four plots (per treatment) at both sites. Soil was washed from the roots under running tapwater. Crown roots were sampled at random, and stored in SDW at 5°C until plating. Twelve 1.0 cm root segments from each replicate were plated on isolation medium (RA) as described in the General Methods. However, roots were not surface-sterilised in NaOCl prior to plating, but were washed three times in SDW, for reasons that will be explained in the Results (Section 9.3). The identity and frequency of fungi infecting these roots was determined.

Crown root samples collected from non-fumigated plots at Mannum on July 25, August 1 and 16 and September 15 were plated on VP_3 medium (as described in the General Methods) to detect *Pythium* spp. within the roots. Twelve, randomly selected, 1.0 cm root segments were plated for each replicate on these dates.

Roots from fumigated plots were examined and their appearance compared with that of roots from unfumigated plots.

Occurrence of Pratylenchus neglectus in Crown Roots

Root lesion nematodes (*P. neglectus*) were observed within the crown roots of Machete wheat sampled from untreated plots at Mannum and Caloote on August 1, 16 and 30, 1989. Randomly selected crown roots were stained in lactophenol-cotton blue (as described in the General Methods), and observed microscopically to detect *P. neglectus* and their eggs within root cortices.

Distribution of Machete Wheat Roots and Symptoms With Depth

The distribution of Machete wheat seminal and crown roots in the soil was investigated at the Mannum and Caloote sites in late August-early September, 1989. Whole plants were removed from fumigated and untreated areas. Two replicates of each were sampled at both sites. Soil was excavated to a depth of 30 - 40 cm, sufficient to remove almost the entire root system intact. Soil was carefully washed from the roots

under running tapwater, while the roots were maintained in the positions they held when in the soil, by a series of metal spikes on a backing plate.

Roots were investigated and cut into segments roughly corresponding to the horizons of the soil profile. The intervals were chosen to correspond to the depth of seeding (5 cm), the maximum depth of cultivation (9 cm), the boundary of the A and B horizons (15 cm) and the boundary between the B_1 and B_2 horizons (23 cm). The B_2 horizon is a heavy clay loam, and remarkably few roots grew into that layer. Crown roots, at the time of sampling, did not penetrate below 23 cm.

The incidence of rotting and lesioning within each depth interval was measured as millimetres of symptoms per plant. Symptoms were divided into two categories: those attributable to *Gaeumannomyces graminis* (which are readily observed as black, stelar lesions), and general cortical rotting and lesions of an indeterminate nature.

The distribution of seminal and crown roots (as dry root weight per plant at each depth interval), and symptoms thereon, from untreated plots of Machete was compared to that of roots from fumigated plots.

9.3 RESULTS

Symptoms on the Crown Roots

The crown roots of plants sampled from untreated plots at both Mannum and Caloote suffered extensive damage, and the progressive deterioration of these roots is demonstrated in Plates 9.1 - 9.3. Crown roots began to grow in mid-July, and by July 25 these roots had started to show signs of cortical discolouration (Plate 9.1A). Cortical destruction progressed through August (Plates 9.1B, 9.2, 9.3A, 9.4A) until, by September 15 (Plate 9.3B), a large proportion of the crown root system was virtually devoid of cortical tissue. Consequently, root hairs were not seen on the rotted areas. Lateral roots were absent from the majority of roots sampled on August 30 (Plate 9.3A), or had been reduced to short, rotted "stumps" or "spikes". It was rare to find a plant with even a single healthy crown root, and only short sections of root remained intact by the final sample date on September 15 (Plate 9.3B).

PLATE 9.1: Crown roots of Machete wheat sampled from non-fumigated plots on July 25 (**A**) and August 1 (**B**), 1989. Small areas of cortical discolouration were visible (➡) on July 25. By August 1, orange-brown lesions had developed, and decortication (➡) had begun.

Plate 9.1

A

B

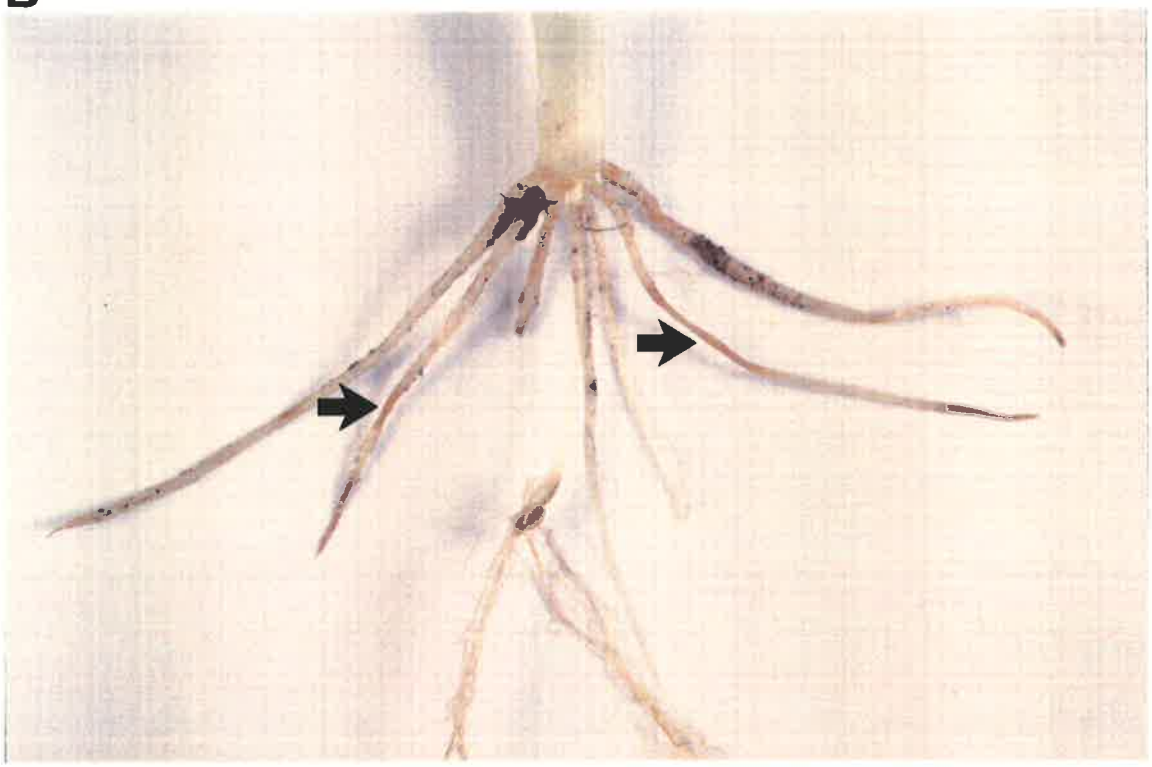


PLATE 9.2: Crown roots of Machete wheat sampled from non-fumigated plots on August 9 (**A**) and August 21 (**B**), 1989. By August 9, crown roots had produced some lateral branches (➡), but lesioning and decortication had progressed since the previous sample date (Plate 9.1). More laterals were present on August 21 (➡). Large sections of root were lesioned and decorticated, but some new crown roots had formed, and these were undamaged.

Plate 9.2

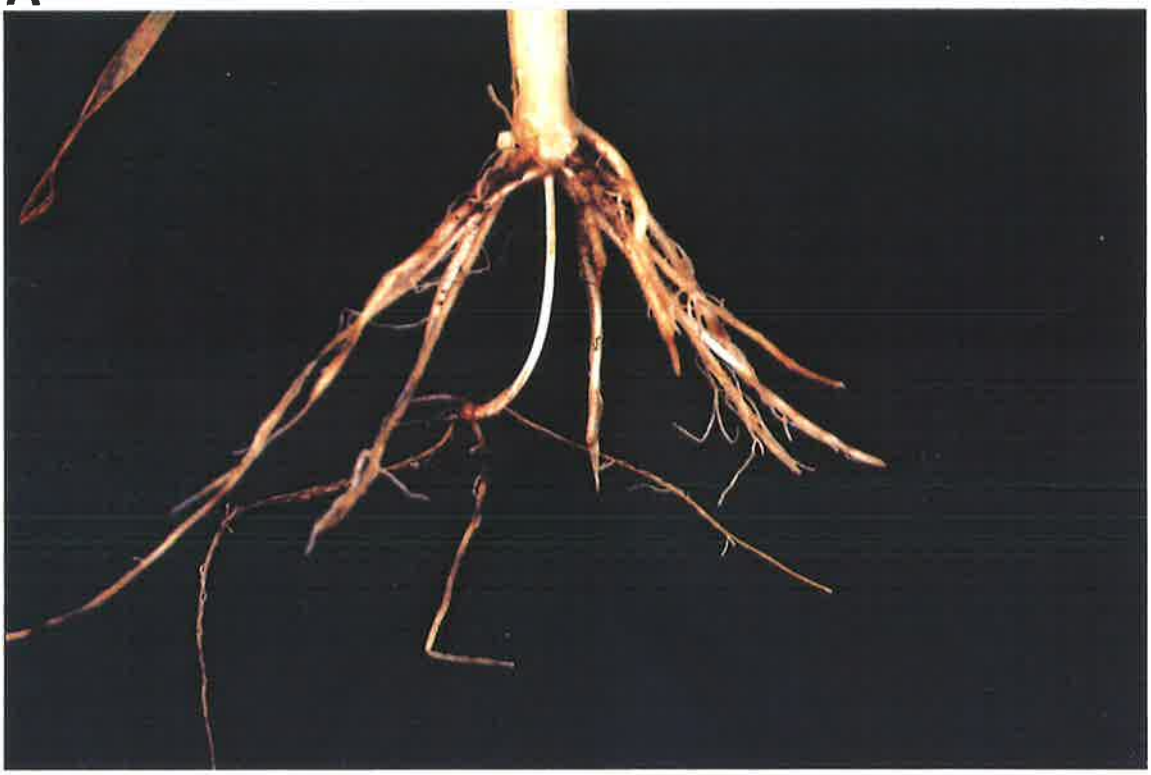
A

B

PLATE 9.3: Crown roots of Machete wheat sampled from non-fumigated plots on August 30 (**A**) and September 15 (**B**), 1989. On August 30, all roots were extensively lesioned and decorticated, with very few lateral root branches. On September 15, only the few intact sections of cortex possessed laterals (➡).

Plate 9.3

A



B

New crown roots, or laterals on the few segments of intact cortex, were sometimes produced in response to late winter-early spring rainfall. These roots grew rapidly, but were soon subject to the same damage as that experienced by adjacent, older crown roots.

Crown roots on plants from fumigated plots were produced in early July. These roots did not display extensive cortical rotting, nor was lateral root growth retarded (Plate 9.4B). Roots from fumigated plots did, to some extent, develop similar symptoms to those from non-fumigated soil, although the damage was never widespread over the root system, and symptom development was delayed until mid-September. Fungi were isolated from crown roots on fumigated plots (Tables 9.3, 9.4), although mostly at lower frequencies than from non-fumigated plots (Tables 9.1, 9.2), especially early in the growing season. Fungi tended to re-colonise fumigated areas, so isolation frequencies were higher later in the growing season (late August to mid-September) than they were previously.

Root damage tended to be more severe at Mannum than at Caloote, which was reflected in the higher overall frequency with which some of the fungi (namely *Fusarium graminearum*, *Microdochium bolleyi*, *Bipolaris sorokiniana* and *Cladosporium* sp.) were isolated from non-fumigated plots at this site (Tables 9.1, 9.2). However, all other species identified were isolated more often (overall) from crown roots at Caloote than from those at Mannum.

Isolation of Fungi - Non-Fumigated Plots

Rhizoctonia solani - Hyphae of this species were sometimes observed on root surfaces prior to the development of symptoms, but subsequently *R. solani* was isolated infrequently. *R. solani* was isolated from 15 - 17% of crown roots at Mannum between early and late August, but by mid-September this species was associated with only 3% of crown roots (Table 9.2). The frequency of *R. solani* also decreased at Caloote: from 39% in late July to only 5 - 8% in mid-August to mid-September (Table 9.1). *R. solani* infected significantly ($P<0.05$) more roots at Caloote (Table 9.1) on July 25 than at any other time over the season. At Mannum, this species infected significantly ($P<0.05$)

PLATE 9.4: **A.** Lesioned and decorticated Machete wheat crown roots from plants sampled on August 30 (Plate 9.3A), 1989.

B. Crown roots of Machete wheat sampled on August 9, 1989, from an area treated with methyl bromide soil fumigant. The subcrown internode and seminal roots were removed to enhance the clarity of the photograph. These crown roots were not lesioned or decorticated, and their "matted" appearance indicates extensive lateral root growth. Compare with Plates 9.1 - 9.3.

Plate 9.4

A

B

fewer roots in September than it did in August (Table 9.2).

The detection of *R. solani* can sometimes be difficult due to the isolation techniques employed. Exposing roots to surface-sterilants, such as sodium hypochlorite (NaOCl), can result in the death of hyphae and consequent failure to isolate the fungus. In early July, two sets of root samples were prepared: one with and one without treatment in 2.5% NaOCl for 60 seconds. The results of this test showed that sterilisation in 2.5% NaOCl (the standard method of treating root samples in all other experiments) virtually eradicated *R. solani* from subsequent isolations. Surface-sterilised roots yielded 0 - 2% *R. solani* when plated on isolation medium, whereas 20% of untreated roots were infected with *R. solani*. Consequently, results are presented for samples not subjected to treatment with NaOCl, and roots were washed in SDW only, so the occurrence of *R. solani* was not underestimated. As roots were not surface-sterilised, it is possible that many isolates represented hyphae, growing saprophytically, external to the roots, rather than those that were actually growing within the root tissues. The frequency of *R. solani* in crown root tissues may therefore have been lower than that actually recorded. The frequency of *R. solani* measured after surface-sterilisation may be an indication of that actually growing within the tissues, and thus an estimate of the frequency of pathogenic isolates of this fungus.

Fusarium spp. - Frequency of *F. graminearum* was recorded separately, as it is more damaging to cereals than the other species of fusaria. Records of other *Fusarium* spp. included isolates of *F. equiseti* and *F. acuminatum*, with lower numbers of *F. oxysporum* and *F. culmorum*. Isolates of these species were positively identified by Professor L. W. Burgess, University of Sydney.

Fusarium spp. represented the bulk of the fungal population isolated, and the frequency of these species increased over the growing season. At Mannum, 61% of crown roots were infected in late July, and 71% by mid-September (Table 9.2). *Fusarium* spp. infected 50% of crown roots at Caloote in late July, and this increased to 94% by mid-September (Table 9.1). The incidence of *Fusarium* spp. increased significantly ($P<0.05$) over the season at Caloote (Table 9.1), but at Mannum (Table 9.2) the incidence of *Fusarium* spp. only increased significantly ($P<0.05$) between July and mid-August.

TABLE 9.1: Frequency of fungi (% root segments infected) isolated from crown roots of Machete wheat plants sampled from untreated plots at Caloote over the 1989 growing season. (Values are the mean of four replicates at each sample date). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations. Species of fungi recorded are: Fus = *Fusarium* spp. (other than *F. graminearum*); Fg = *F. graminearum*; Mb = *Microdochium bolleyi*; Bs = *Bipolaris sorokiniana*; Rs = *Rhizoctonia solani*; Gg = *Gaeumannomyces graminis*; Clad = *Cladosporium* sp.; Alt = *Alternaria alternata*; Uat = *Ulocladium atrum*; Emb = *Embellisia chlamydospora*; Curv = *Curvularia inaequalis*.

	SPECIES OF FUNGI ISOLATED FROM CROWN ROOTS										
Date	**Fus**	**Fg**	**Mb**	**Bs**	**Rs**	**Gg**	**Clad**	**Alt**	**Uat**	**Emb**	**Curv**
25.7.89	50.0	11.1	0	0	38.9	0	5.6	0	0	0	0
01.8.89	51.3	9.4	6.8	4.6	20.2	6.8	7.7	3.1	0	15.3	0
09.8.89	78.6	7.1	0	0	0	0	0	0	0	0	0
16.8.89	87.5	16.7	4.2	4.2	8.3	0	4.2	0	8.3	0	0
21.8.89	92.5	5.1	5.8	1.5	6.7	3.3	6.5	9.0	10.0	11.2	0
30.8.89	82.2	2.2	23.4	2.4	4.6	6.0	1.9	13.2	11.6	11.5	0
15.9.89	93.8	5.6	2.1	2.1	8.3	6.3	2.8	13.9	4.2	9.7	0
25.7.89	(45.0)	(18.7)	(10.0)	(10.0)	(38.2)	(10.0)	(14.4)	(10.0)	(10.0)	(10.0)	(10.0)
01.8.89	(45.9)	(16.9)	(15.4)	(13.8)	(25.8)	(15.4)	(16.5)	(12.7)	(10.0)	(22.6)	(10.0)
09.8.89	(62.4)	(15.5)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)
16.8.89	(69.3)	(24.1)	(11.8)	(11.8)	(16.7)	(10.0)	(11.8)	(10.0)	(16.7)	(10.0)	(10.0)
21.8.89	(74.4)	(14.3)	(13.5)	(10.0)	(15.3)	(11.5)	(14.9)	(17.0)	(18.2)	(19.3)	(10.0)
30.8.89	(65.2)	(11.8)	(28.9)	(10.2)	(13.1)	(14.4)	(10.5)	(20.6)	(19.1)	(18.6)	(10.0)
15.9.89	(75.1)	(14.2)	(9.6)	(9.6)	(17.0)	(14.9)	(10.7)	(21.8)	(12.6)	(18.1)	(10.0)
LSD(0.05)	19.5	9.9	6.3	4.2	19.3	7.2	6.6	6.6	5.8	7.5	-

TABLE 9.2: Frequency of fungi (% root segments infected) isolated from crown roots of Machete wheat plants sampled from untreated plots at Mannum over the 1989 growing season. (Values are the mean of four replicates at each sample date). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations. Species of fungi recorded are the same as those listed in Table 9.1, with the addition of Py = *Pythium* spp.

	SPECIES OF FUNGI ISOLATED FROM CROWN ROOTS											
Date	**Fus**	**Fg**	**Py**	**Mb**	**Bs**	**Rs**	**Gg**	**Clad**	**Alt**	**Uat**	**Emb**	**Curv**
25.7.89	61.1	22.2	41.7	0	0	0	0	0	0	0	0	0
01.8.89	51.5	18.6	45.8	11.9	5.0	17.3	0	6.2	0	0	11.4	0
09.8.89	...	...	...	...	...	...	...	...	...	...	...	...
16.8.89	83.3	83.3	70.8	30.0	23.3	10.0	3.3	3.3	3.3	0	3.3	0
21.8.89	76.7	25.0	...	19.8	8.1	15.5	0	15.8	5.3	4.3	6.4	4.1
30.8.89	...	...	...	...	...	...	...	...	...	...	...	...
15.9.89	70.6	33.9	87.5	6.7	3.3	2.8	0	2.8	11.1	6.7	1.7	0
25.7.89	(51.5)	(26.8)	(39.8)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)
01.8.89	(45.8)	(24.6)	(42.6)	(19.5)	(14.2)	(24.0)	(10.0)	(15.0)	(10.0)	(10.0)	(18.1)	(10.0)
09.8.89	...	...	...	...	...	...	...	...	...	...	...	...
16.8.89	(65.9)	(65.9)	(58.2)	(33.2)	(28.9)	(18.4)	(10.5)	(10.5)	(10.5)	(10.0)	(10.5)	(10.0)
21.8.89	(61.4)	(29.8)	...	(24.8)	(15.8)	(22.0)	(10.0)	(22.7)	(13.9)	(12.8)	(15.1)	(11.7)
30.8.89	...	...	...	...	...	...	...	...	...	...	...	...
15.9.89	(58.4)	(34.9)	(69.7)	(15.3)	(11.5)	(10.7)	(10.0)	(10.7)	(19.3)	(15.3)	(8.9)	(10.0)
LSD(0.05)	11.7	17.0	15.7	13.5	7.3	7.2	-	8.0	6.1	3.6	12.1	1.4

Fusarium graminearum - At Caloote, *F. graminearum* infected 2 - 17% of crown roots, but tended to decrease in frequency over the growing season (Table 9.1). *F. graminearum* was more common at Mannum (Table 9.2) than at Caloote (Table 9.1). This species infected 19 - 34% of crown roots at Mannum, although the frequency in mid-August was unusually high (83%). The incidence of *F. graminearum* at Caloote did not increase significantly over the season. At Mannum, with the exception of the high value recorded on August 16, *F. graminearum* did not increase significantly in frequency.

Microdochium bolleyi - At Mannum, the frequency of *M. bolleyi* increased until mid-August, but thereafter decreased until only 7% of roots were infected in mid-September (Table 9.2). *M. bolleyi* was less frequent at Caloote, where its incidence did not increase over the growing season (Table 9.1). The incidence of *M. bolleyi* at Mannum significantly ($P<0.05$) increased between July and mid-August, but thereafter declined. With the exception of the high value recorded at Caloote on August 30, the frequency of *M. bolleyi* at this site did not significantly increase over the season.

Pythium spp. - The frequency of pythia infecting crown roots was only measured at the Mannum site (Table 9.2). These fungi, next to the fusaria, were the most commonly isolated species. *Pythium* spp. increased in frequency from 42% in late July to 88% in mid-September. The incidence of *Pythium* spp. significantly ($P<0.05$) increased over the growing season.

Gaeumannomyces graminis - *G. graminis* rarely infected crown roots at either site. At Mannum (Table 9.2) only 0 - 3% of roots were infected, and only 0 - 7% at Caloote (Table 9.1). The incidence of this species did not change significantly over the growing season.

Bipolaris sorokiniana - This species was isolated infrequently from crown roots. Less than 5% of roots were infected at Caloote (Table 9.1). Up to 8% of roots were infected at Mannum (Table 9.2), except in mid-August, when 23% infection was recorded. Apart from the high value recorded at Mannum on August 16, the incidence of *B. sorokiniana* did not change significantly over the season at either site.

Other Species - *Cladosporium* sp., *Alternaria alternata*, *Ulocladium atrum*, *Embellisia chlamydospora* and *Curvularia inaequalis* were isolated sporadically

throughout the growing season, with none exceeding 16% infection, and most at much lower frequencies (Tables 9.1, 9.2).

Isolation of Fungi - Fumigated Plots

With the exception of *F. graminearum*, *Cladosporium* sp. and *A. alternata*, the overall frequency of fungi at Caloote was lower on fumigated (Table 9.3) than on non-fumigated (Table 9.1) plots. At Mannum, fumigation reduced the overall frequency of all species (Table 9.4). The fusaria, however, seemed to be affected little by the fumigation treatment. All other species occurred at low levels, often increasing in frequency towards the end of the growing season, as they re-colonised the treated soil.

Occurrence of Pratylenchus neglectus in Crown Roots

The root lesion nematode, *P. neglectus*, was identified in the undamaged portions of crown root cortices (Plate 9.5A), adjacent to lesioned or rotted areas. On August 1 and 16, *P. neglectus* were detected in all 25 crown roots examined. Thirty eight roots were examined on August 30, and all but eight of these contained nematodes, and the severity of root rotting was much greater than that observed earlier in August. Nematode numbers increased between August 1 and 16. However, on August 30, fewer nematodes infected roots than were detected on either August 1 or 16. No eggs were present in the roots examined on August 1. Numerous eggs were detected on August 16 (Plate 9.5A), but few were seen on August 30.

Equivalent examinations of crown roots from fumigated areas failed to detect any *P. neglectus*, until August 16, when nematodes were occasionally seen in groups within the cortex. One such crown root contained 54 nematodes and eighteen eggs.

When examining roots, it was also noted that roots from non-fumigated areas that contained *P. neglectus* had poor lateral root development compared to those from fumigated areas that had produced extensive laterals. Nematodes were detected in lateral root cortices, as well as in those of the main root axes (Plate 9.5B).

Numerous *Pythium* oospores were seen in the stained crown root samples from both sites. Although the frequency of *Pythium* infection was not recorded at Caloote

TABLE 9.3: Frequency of fungi (% root segments infected) isolated from crown roots of Machete wheat plants sampled from fumigated plots at Caloote over the 1989 growing season. (Values are the mean of four replicates at each sample date). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations. Species of fungi recorded are the same as those listed in Table 9.1.

	SPECIES OF FUNGI ISOLATED FROM CROWN ROOTS										
Date	**Fus**	**Fg**	**Mb**	**Bs**	**Rs**	**Gg**	**Clad**	**Alt**	**Uat**	**Emb**	**Curv**
25.7.89	75.0	0	0	0	0	0	0	0	0	0	0
01.8.89	57.3	5.6	0	0	9.8	2.8	12.7	0	0	0	0
09.8.89	31.8	13.6	0	0	0	0	13.6	0	0	0	0
16.8.89	50.0	58.3	0	0	4.2	0	0	12.5	0	0	0
21.8.89	71.5	27.8	1.4	1.4	0	0	18.4	9.0	4.2	0	0
30.8.89	37.5	45.8	0	0	0	0	13.9	38.9	1.4	5.6	0
15.9.89	88.9	25.0	22.2	0	2.8	0	8.3	36.1	0	2.8	0
25.7.89	(60.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)
01.8.89	(49.3)	(14.5)	(10.0)	(10.0)	(18.1)	(12.4)	(20.6)	(10.0)	(10.0)	(10.0)	(10.0)
09.8.89	(34.3)	(21.6)	(10.0)	(10.0)	(10.0)	(10.0)	(21.6)	(10.0)	(10.0)	(10.0)	(10.0)
16.8.89	(45.0)	(49.8)	(10.0)	(10.0)	(11.8)	(10.0)	(10.0)	(20.7)	(10.0)	(10.0)	(10.0)
21.8.89	(58.4)	(29.3)	(10.9)	(11.0)	(10.0)	(10.0)	(24.6)	(17.1)	(13.3)	(10.0)	(10.0)
30.8.89	(37.5)	(41.7)	(10.0)	(10.0)	(10.0)	(10.0)	(21.4)	(37.6)	(10.9)	(14.5)	(10.0)
15.9.89	(75.0)	(30.0)	(27.1)	(10.0)	(10.7)	(10.0)	(16.9)	(36.9)	(10.0)	(10.7)	(10.0)
LSD(0.05)	18.3	26.0	6.0	1.0	5.6	2.7	8.8	12.6	2.6	4.0	-

TABLE 9.4: Frequency of fungi (% root segments infected) isolated from crown roots of Machete wheat plants sampled from fumigated plots at Mannum over the 1989 growing season. (Values are the mean of four replicates at each sample date). Values in brackets are arcsine √% transformed means, and the LSD (0.05) is based on these transformations. Species of fungi recorded are the same as those listed in Table 9.1.

	SPECIES OF FUNGI ISOLATED FROM CROWN ROOTS										
Date	**Fus**	**Fg**	**Mb**	**Bs**	**Rs**	**Gg**	**Clad**	**Alt**	**Uat**	**Emb**	**Curv**
25.7.89	60.0	0	0	0	0	0	0	0	0	0	0
01.8.89	46.2	2.5	17.5	0	13.0	2.8	2.5	0	0	3.8	0
09.8.89	...	...	...	...	...	...	...	...	...	...	...
16.8.89	58.3	41.7	0	0	0	0	12.5	4.2	0	0	0
21.8.89	64.3	27.0	11.9	0	0	0	24.7	16.4	6.6	3.3	1.5
30.8.89	...	...	...	...	...	...	...	...	...	...	...
15.9.89	38.9	63.9	8.3	0	2.8	0	2.8	2.8	0	0	0
25.7.89	(50.8)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)	(10.0)
01.8.89	(42.1)	(12.1)	(24.1)	(10.0)	(20.4)	(12.4)	(12.1)	(10.0)	(10.0)	(13.3)	(10.0)
09.8.89	...	...	...	...	...	...	...	...	...	...	...
16.8.89	(49.8)	(40.2)	(10.0)	(10.0)	(10.0)	(10.0)	(20.7)	(11.7)	(10.0)	(10.0)	(10.0)
21.8.89	(53.7)	(29.2)	(19.6)	(10.0)	(10.0)	(10.0)	(27.8)	(23.8)	(15.4)	(11.6)	(9.8)
30.8.89	...	...	...	...	...	...	...	...	...	...	...
15.9.89	(38.2)	(53.8)	(16.7)	(10.0)	(10.7)	(10.0)	(10.7)	(10.7)	(10.0)	(10.0)	(10.0)
LSD(0.05)	16.8	16.2	7.6	-	7.2	3.3	7.6	2.3	2.8	4.8	1.4

PLATE 9.5: The root lesion nematode, *Pratylenchus neglectus*, was detected in the intact sections of crown root cortices, adjacent to lesioned and decorticated areas.

A. *P. neglectus* plus eggs (x400 magnification).

B. *P. neglectus* in the cortex of main root axis and associated lateral root (x100 magnification).

Plate 9.5

A

B

(Table 9.1) it was probably of the same order as that recorded from the Mannum site (Table 9.2).

Distribution of Machete Wheat Roots and Symptoms With Depth

Fumigation enhanced the growth of crown roots at both Mannum (Figure 9.1) and Caloote (Figure 9.2), especially in the top 9.0 cm (the cultivated layer) of the soil. However, this was only significant ($P<0.05$) at the Mannum site. The growth of seminal roots at Mannum was enhanced by fumigation, especially below a depth of 9 cm (Figure 9.5). The same was true at Caloote (Figure 9.6), although root weight on fumigated plots at this site decreased again below 15 cm. Due to substantial variation between replicates, the effect of fumigation on seminal root growth was not statistically significant at either site. Growth of Machete wheat on fumigated and non-fumigated plots is demonstrated in Plate 9.6.

The incidence of symptoms on crown roots decreased with depth. Fumigation significantly ($P<0.05$) alleviated root damage at all depths at Mannum (Figure 9.3) but only at 0 - 5 cm and at 9 - 15 cm at Caloote (Figure 9.4). The majority of rotting occurred in the top 5.0 cm of the soil. The extent of rotting on untreated soil was three to four times that on fumigated soil. Fumigation significantly ($P<0.05$) reduced the incidence of symptoms on seminal roots at Mannum, but only at 0 - 5 cm (Figure 9.7). Below 9 cm, few symptoms were recorded. At Caloote, the incidence of symptoms on the seminal roots increased with depth, and fumigation significantly ($P<0.05$) alleviated the occurrence of these symptoms at both 15 - 23 cm and 23 - 34 cm (Figure 9.8).

Lesions attributable to *G. graminis* were rare on both seminal and crown roots of plants from untreated areas, and mainly occurred in the top 9.0 cm of the soil (Table 9.5). Crown roots from Mannum showed more symptoms of *G. graminis* infection than did those from Caloote. However, *G. graminis* was isolated rarely from crown roots at either site, but was recorded more frequently at Caloote (Table 9.1) than at Mannum (Table 9.2). Although incidence of black, stelar lesions on the crown roots decreased with depth, a significantly ($P<0.05$) higher value was recorded between 5.0 and 9.0 cm at Mannum. Incidence of lesions on the crown roots did not significantly change with depth

FIGURE 9.1: Distribution of Machete wheat crown roots (mg/plant) with depth (cm). Plants sampled from fumigated (FUM) and untreated (NON-FUM) plots at Mannum in late August, 1989. (Values are the mean of two replicates). LSD (0.05) = 82.6.

FIGURE 9.2: Distribution of Machete wheat crown roots (mg/plant) with depth (cm). Plants sampled from fumigated (FUM) and untreated (NON-FUM) plots at Caloote in early September, 1989. (Values are the mean of two replicates). LSD (0.05) = 31.9.

FIGURE 9.3: Frequency of rotting and lesioning (mm/plant), other than that attributable to *Gaeumannomyces graminis*, with depth (cm) on crown roots of Machete wheat sampled from fumigated (FUM) and untreated (NON-FUM) plots at Mannum in late August, 1989. (Values are the mean of two replicates). LSD (0.05) = 5.4.

FIGURE 9.4: Frequency of rotting and lesioning (mm/plant), other than that attributable to *Gaeumannomyces graminis*, with depth (cm) on crown roots of Machete wheat sampled from fumigated (FUM) and untreated (NON-FUM) plots at Caloote in early September, 1989. (Values are the mean of two replicates). LSD (0.05) = 2.6.

Figure 9.1

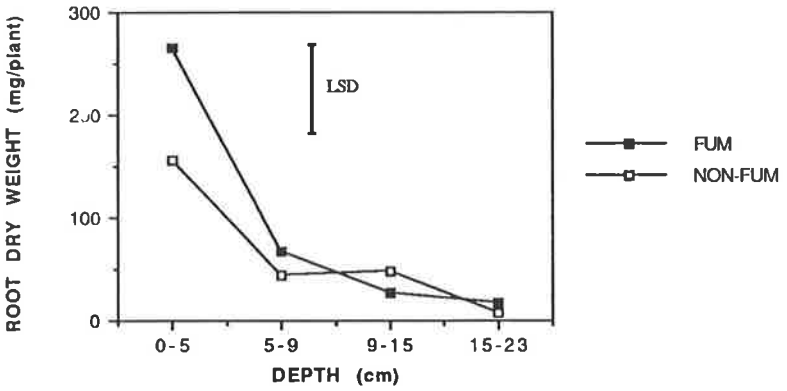


Figure 9.2

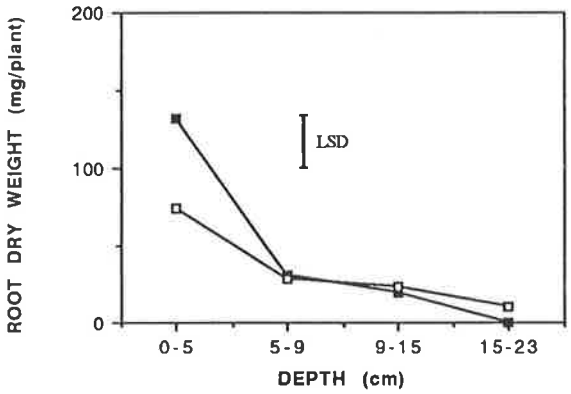


Figure 9.3

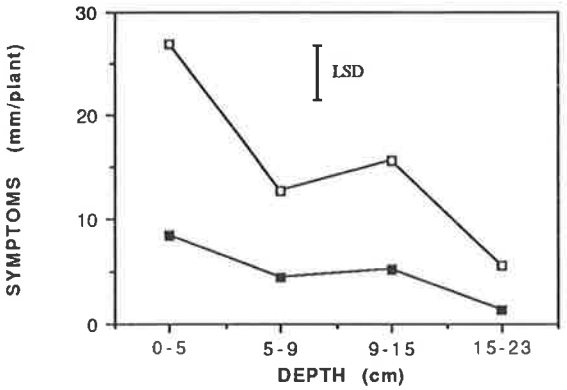


Figure 9.4

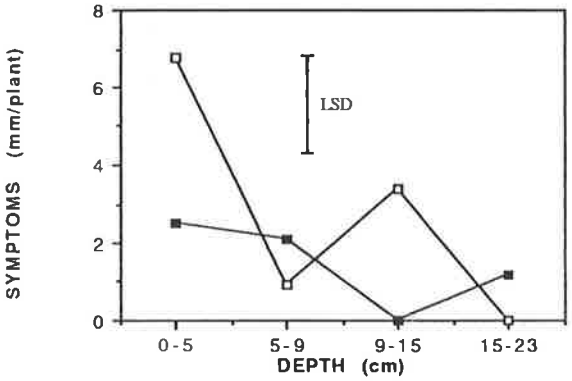


FIGURE 9.5: Distribution of Machete wheat seminal roots (mg/plant) with depth (cm). Plants sampled from fumigated (FUM) and untreated (NON-FUM) plots at Mannum in late August, 1989. (Values are the mean of two replicates). LSD (0.05) = 112.5.

FIGURE 9.6: Distribution of Machete wheat seminal roots (mg/plant) with depth (cm). Plants sampled from fumigated (FUM) and untreated (NON-FUM) plots at Caloote in early September, 1989. (Values are the mean of two replicates). LSD (0.05) = 80.2.

FIGURE 9.7: Frequency of rotting and lesioning (mm/plant), other than that attributable to *Gaeumannomyces graminis*, with depth (cm) on seminal roots of Machete wheat sampled from fumigated (FUM) and untreated (NON-FUM) plots at Mannum in late August, 1989. (Values are the mean of two replicates). LSD (0.05) = 47.3.

FIGURE 9.8: Frequency of rotting and lesioning (mm/plant), other than that attributable to *Gaeumannomyces graminis*, with depth (cm) on seminal roots of Machete wheat sampled from fumigated (FUM) and untreated (NON-FUM) plots at Caloote in early September, 1989. (Values are the mean of two replicates). LSD (0.05) = 27.1.

Figure 9.5

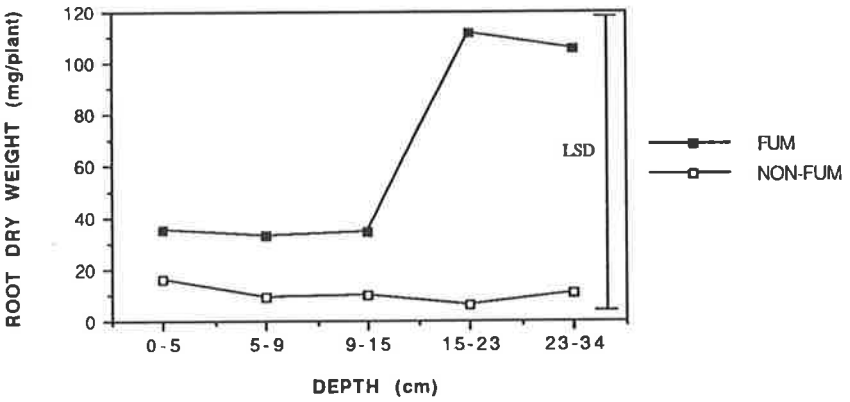


Figure 9.6

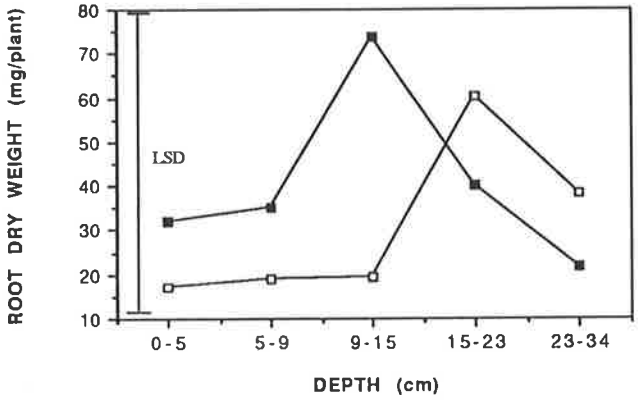


Figure 9.7

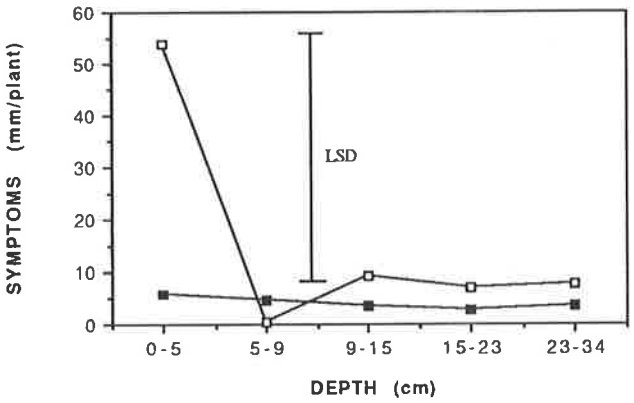


Figure 9.8

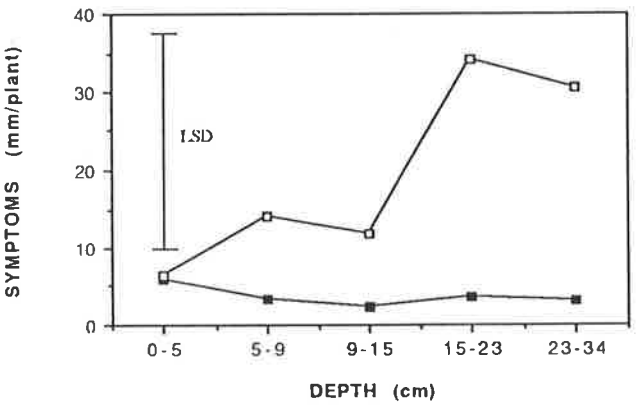


PLATE 9.6: Root and shoot growth of Machete wheat sampled from non-fumigated (left) and fumigated (right) plots at Caloote in August, 1989.

Plate 9.6

at Caloote, but the incidence of these lesions did tend to decrease with depth. The incidence of these lesions on the seminal roots significantly ($P<0.05$) decreased with depth at both sites.

TABLE 9.5: Incidence of symptoms attributed to *Gaeumannomyces graminis* (mm of lesions/plant) with depth on the seminal (Srt) and crown (Crt) roots of Machete wheat sampled from untreated plots at Mannum and Caloote in late August-early September, 1989. (Values are the mean of two replicates).

	MANNUM		CALOOTE	
Depth (cm)	**Srt**	**Crt**	**Srt**	**Crt**
0 - 5	3.3	6.3	15.2	2.5
5 - 9	8.4	16.5	3.9	2.1
9 - 15	0.3	4.1	0	0
15 - 23	0	0.5	0	1.2
23 - 34	0.3	...	0.3	...
LSD (0.05)	6.6	6.7	6.8	4.7

9.3.1 Summary of Results

Studies on the damage incurred by crown roots over the 1989 growing season showed that:

(1) The crown roots of wheat plants deteriorate rapidly, with extensive decortication and a notable lack of lateral roots and root hairs.

(2) Many species of fungi were isolated from diseased crown roots, but *Fusarium* and *Pythium* spp. were the only fungi to significantly increase in frequency as the severity of damage increased. The occurrence of *M. bolleyi*, to some extent, also increased, but only early in the growing season.

(3) Frequency of *R. solani* significantly decreased over the season, while the severity of root damage increased. Although the symptoms observed on the roots were similar to those that have been associated, by some workers, with infection by *R.*

solani, this fungus did not appear to contribute to crown root damage.

(4) The root lesion nematode, *P. neglectus*, was identified in lesioned crown roots.

9.4 DISCUSSION

The crown roots of Machete wheat sustained extensive damage, so that the extent to which they grew through the soil was restricted. Crown root growth was retarded most in the top 9.0 cm of soil, and the severity of symptoms was greatest in the top 5.0 cm. Warcup (1957) found that the frequency of fungi in a South Australian wheat field soil decreased with depth, and significantly fewer isolates were obtained at 15.0 cm than at 2.5 cm or 8.0 cm depth. The extent of crown root damage at Mannum and Caloote in 1989 followed this pattern, with fewer lesions and rotted sections occurring below 9.0 cm depth.

Pythium and *Fusarium* spp. (other than *F. graminearum*) were the only fungi to significantly increase in frequency as the severity of root damage increased, and are therefore candidates for inciting the disease. The incidence of *Microdochium bolleyi* increased early in the season but, unlike the pythia and fusaria, decreased in frequency later in the growing season. *Rhizoctonia solani* infected a moderate proportion of roots sampled in late July and early August, but thereafter the frequency of this species declined significantly. Apart from the unusually high frequency of *F. graminearum* at Mannum in mid-August, this species (unlike the remainder of the *Fusarium* population in the roots) did not significantly increase in occurrence as the severity of damage increased. Other species (*Bipolaris sorokiniana*, *Gaeumannomyces graminis*, *Cladosporium* sp., *Alternaria alternata*, *Ulocladium atrum*, *Embellisia chlamydospora* and *Curvularia inaequalis*) were isolated sporadically and at low frequencies, and therefore probably did not contribute significantly to the crown root damage observed.

Fusarium and *Pythium* spp. have been associated with the poor growth of lateral roots (Chapter 6), and with rotting of the root cortex. Salt (1977) reported that large numbers of *P. arrhenomanes* oospores in cereal roots were accompanied by browning and collapse of cortical tissues. *Pythium* oospores were also detected in rotted wheat

roots with a tapering, brown tip (Salt, 1971). Numerous *Pythium* oospores were detected in rotted crown roots sampled from Mannum and Caloote in 1989, and roots suffered damage identical to that described by Salt (1971, 1977). Furthermore, *Pythium* spp. were isolated from a large proportion of root samples from the Mannum site.

In pathogenicity tests, Fedel-Moen and Harris (1987) found that *F. acuminatum*, *F. equiseti* and *F. oxysporum* caused discolouration and sloughing of cortical tissues, and the more pathogenic isolates caused extensive decortication. These observations were made on oat and barley seminal roots, but the reaction of wheat crown roots to these fungi may be similar. The three *Fusarium* spp. tested by Fedel-Moen and Harris (1987) are those most frequently isolated from cereals in South Australia (Harris, 1986; Fedel-Moen and Harris, 1987).

F. graminearum causes "crown rot" of cereals, which is characterised by dark brown crown tissues and honey-brown discolouration of the stem base (Burgess *et al.*, 1981). However, "crown rot" is more severe in New South Wales (Burgess *et al.*, 1981) than it is in South Australia (Mayfield, 1981). This species does not usually contribute to root rot (Burgess *et al.*, 1984), although proximal regions of seminal and crown roots are often colonised by *F. graminearum* (Burgess *et al.*, 1981). This would explain the higher level of *F. graminearum* recorded on crown roots in 1989 than was recorded on seminal roots in the same year (Chapter 10). Conversely, *M. bolleyi* was isolated from crown roots in 1989 at lower frequencies (average 9%) than from seminal roots in 1987 (average 26%; Chapter 4). However, the incidence of *M. bolleyi* on crown roots in 1989 was similar to that isolated from seminal roots in 1988 and 1989 (8 - 10%; Chapter 8).

Besides *Pythium* and other *Fusarium* spp., *F. graminearum* may contribute to crown root damage observed at Mannum and Caloote, although the frequency of isolation did not significantly increase as the severity of crown root rotting increased. *M. bolleyi* seems to infect crown roots less than seminal roots (in some years) but, as the isolation frequency significantly increased early in the season, *M. bolleyi* may also be added to the list of potential contributors to crown root rotting early in the growing season.

Some of the symptoms observed on the crown roots were consistent with those that have been attributed to *R. solani* infection of seminal roots: orange-brown cortical

discolouration, decortication, shortened and stiffened roots, "spear" tips where roots are tapered with dead tips and exposed stele (Samuel and Garrett, 1932; Wiese, 1987). Samuel and Garrett (1932) described *R. solani* as the cause of a "definite seedling disease" of wheat, oats and barley on mallee soils in South Australia. In fact, they stated that severe infection of seminal roots prevented seedlings from producing any crown roots. The role of *R. solani* as a seedling disease is further supported by the observation that this fungus is more active at low soil temperatures (Samuel and Garrett, 1932).

The frequency of *R. solani* significantly decreased over the growing season as crown root damage increased in severity. Pathologists have long recognised the difficulty in isolating *R. solani* from rotted roots once plants are past the seedling stage. Samuel and Garrett (1932) and Hynes (1937a) noted the transitory nature of *R. solani* in root tissues, and Harris and Moen (1985a, 1985b) later showed that secondary parasites supplanted the initial *R. solani* infection of roots. The diverse range of associated organisms cause more damage than *R. solani* alone, and these secondary organisms may be the ultimate cause of crop loss (Harris and Moen, 1985b).

R. solani, when acting alone as a pathogen, has always been associated with diseased plants that occur in more or less circular patches (Samuel and Garrett, 1932; Hynes, 1937a; Kerr, 1955; Butler, 1962; de Beer, 1965; Scott *et al.*, 1979; Deacon and Scott, 1985; Roberts and Sivasithamparam, 1986; Wiese, 1987). These patches are of a constant nature, reappearing in the same position each year (Kerr, 1955). For patches to occur in the field, a local increase in the population of pathogenic strains is necessary (Kerr, 1955; de Beer, 1965). Below these critical levels, the population of *R. solani* is too low to cause appreciable disease (Kerr, 1955). However, plants sampled in 1989 were not taken from patches of poor growth, and were representative of the health of plants throughout the whole crop. Field symptoms of the *Rhizoctonia* "bare patch" disease were absent from sites investigated at Mannum and Caloote.

Kerr (1955) showed that 70% of *R. solani* isolates from within patches were pathogenic to wheat, but only 12.5% from outside the patches were pathogenic. De Beer (1965) also noted that, in South Australia, pathogenic isolates occurred more frequently inside than outside patches. Roberts and Sivasithamparam (1986) reported the same

finding from Western Australia. Isolation of *R. solani* from field samples is no guarantee that it is capable of causing substantial root damage, unless the pathogenicity of isolates is demonstrated. Menzies (1970) commented that *R. solani* is probably "blamed for disease damage in cases where it is only an innocent bystander".

The pathogenicity of *R. solani* isolated from crown roots in 1989 was not tested, but it is reasonable to assume that these isolates were less pathogenic than those that would have been present had patches occurred in the field. Furthermore, when roots were surface-sterilised less than 2% subsequently yielded *R. solani* when plated on isolation medium, whereas 20% of untreated roots were infected with *R. solani*. The higher percentage probably represents the frequency of both pathogenic and non-pathogenic *R. solani* strains, while the lower value indicates the proportion of *R. solani* isolates actually growing parasitically within root tissues. A substantial number of isolates, therefore, were probably growing saprophytically on the root exterior, without causing significant root damage.

For the reasons discussed above, it is doubtful that *R. solani* significantly contributed to the damage incurred by crown roots under the conditions investigated in 1989. Symptoms observed at later growth stages are unlikely to be specifically due to *R. solani* (Harris and Moen, 1985b), and disease caused by this fungus is often diagnosed incorrectly (Harris, 1987). It is thus critical that the role of *R. solani* in root disease is not inferred from the appearance of symptoms alone, especially on the crown roots. Nevertheless, *R. solani* may be an important component of the disease complex, as it infects seedlings (Samuel and Garrett, 1932) and juvenile tissues (Butler, 1962; Moen and Harris, 1985), inciting damage at an early stage of plant growth. Subsequently, rotted roots are invaded by a range of secondary parasites that are more damaging than the initial *R. solani* infection (Harris and Moen, 1985a, 1985b). Detailed isolations, such as those reported here, must therefore be made throughout the growing season, and the species of fungi infecting rotted roots identified and related to the extent of root damage, before symptoms are attributed to a specific organism.

Besides species of *Fusarium* and *Pythium* (and *M. bolleyi* early in the season), the root lesion nematode (*Pratylenchus neglectus*) may be involved in causing the observed

damage to crown roots. Root lesion nematodes occur at depth in the soil (Thompson *et al.*, 1981; Doyle *et al.*, 1987; Thompson and Clewett, 1989), and initially infect the seminal roots. At Caloote, seminal root damage increased with depth, while root growth decreased. *P. neglectus* numbers in roots increase exponentially (Baxter and Blake, 1968), and the nematodes then migrate from damaged tissues into fresh roots to continue feeding (Dropkin, 1989). *P. neglectus* would thus move up the soil profile and infect crown roots as they are produced. These nematodes were observed in the intact regions of crown root cortices, adjacent to lesioned and rotted areas. It is probable that invasion by the nematode initiates damage, which is then exacerbated by fungi. The reverse is unlikely, because fungal infection interferes with the activities of nematodes within root tissues (Mauza and Webster, 1982; Patel, 1983; Walia and Gupta, 1986; Starr *et al.*, 1989; Chandel and Sharma, 1989).

Although *Fusarium* spp. (other than *F. graminearum*), *Pythium* spp. and *M. bolleyi* appear to be likely candidates responsible for causing the damage to crown roots, the incidence of *Fusarium* spp. and *M. bolleyi* was affected little by fumigation. *Pythium* spp. were not controlled by the application of methyl bromide (Chapter 5). Roots on the fumigated areas suffered negligible damage (Plate 9.4B), but the *Fusarium* spp. and, to some extent *M. bolleyi*, were isolated at similar frequencies from the fumigated (Tables 9.3, 9.4) and non-fumigated (Tables 9.1, 9.2) plots. *P. neglectus*, however, was absent from the fumigated areas, at least early in the growing season, suggesting that the nematode also played an important role in causing the damage to crown roots. The role of *P. neglectus* in root disease of wheat, in conjunction with fungi, was therefore investigated further.

CHAPTER 10

THE ROLE OF *PRATYLENCHUS NEGLECTUS* IN ROTTING OF WHEAT ROOTS

10.1 INTRODUCTION

As described in the previous chapter, root lesion nematodes (*Pratylenchus neglectus*, syn. *P. minyus*) were detected in the cortical tissues of wheat roots sampled from the Murray Mallee during the 1989 growing season. These nematodes were positively identified by Dr J. M. Fisher of the Plant Pathology Department, Waite Institute.

In view of this discovery, a second set of field experiments (on a small scale) was planted at two Murray Mallee field sites (Figure 3.1) in the 1989 growing season. The aims of these field trials were to examine the role of these nematodes in the deterioration of root cortices and the failure of lateral roots to grow, and to elucidate the symptoms associated with infection by *P. neglectus*. Two varieties of wheat were grown in areas treated with the nematicide Temik® (aldicarb) to control nematodes, and methyl bromide soil fumigant was applied to control fungi and bacteria as well as nematodes.

P. neglectus has been noted in South Australian cereal crops since 1956 (J. M. Fisher, personal communication), but the role of this nematode in cereal root disease has never been clearly defined. Stynes (1975) found that *P. neglectus* was ubiquitous in wheat crops across the Yorke Peninsula and the Lower North of South Australia, and it has been recognised in most areas of the South Australian cereal belt (de Beer, 1965; Kimpinski, 1972; Kimpinski *et al.*, 1976; Patel, 1983) as well as in Victoria (Price, 1970), Western Australia (J. P. Thompson, personal communication) and some parts of Queensland (Colbran and McCulloch, 1965).

Another species of *Pratylenchus* also occurs on cereals in Australia and elsewhere. *P. thornei* (which is relatively uncommon on cereals in South Australia) is responsible for diminished wheat yields on the Darling Downs of Queensland (Colbran and McCulloch,

1965; Thompson *et al.*, 1981), in northern New South Wales (Doyle *et al.*, 1986, 1987) and in Mexico (Van Gundy *et al.*, 1974). *P. thornei* causes lower leaf chlorosis, stunting of plants and reduced tillering (Van Gundy *et al.*, 1974; Thompson *et al.*, 1981; Doyle *et al.*, 1987). Roots become necrotic (Baxter and Blake, 1968), and yield reductions of 50% are common in northern New South Wales (Doyle *et al.*, 1987). *P. neglectus*, however, has not been shown to be associated with yield reductions in Australia, although it is partly responsible for poor yielding wheat crops in Ontario, Canada (Benedict and Mountain, 1956).

10.2 METHODS

Warigal and Machete wheat were planted, by hand, on the cereal-growing properties of J. Schirmer (Cambrai) and B. Ramm (Mannum) on August 25 and 28, respectively. Sections of the existing wheat crops were removed in mid-August by application of the herbicide Roundup® (glyphosate) or by defoliation. Areas were then treated with methyl bromide soil fumigant (as described in the General Methods for field experiments) or the nematicide Temik® (aldicarb; 2-methyl-2-[methylthio] propionaldehyde o-[methylcarbamoyl] oxime) at the rate of 5.0 kg/ha. Single rows (1.0 m long) of each variety, replicated twice, were planted on the area treated with aldicarb. Two replicate blocks (approximately 6.0 m^2) of each variety were planted on both the fumigated and untreated areas at each site.

Five plants were sampled from each replicate of the three treatments on three dates. Machete and Warigal were sampled at Cambrai on September 25, and on October 17 and 31. Both varieties were sampled from Mannum on October 17. Plants were carefully dug to extract intact root systems, and soil was washed from the roots under running tapwater. Plants were stored in plastic bags at 5°C until processing.

Seedlings were spread on a white tray, in distilled water, to enable symptoms on the roots to be described. At the same time, the following measurements were recorded for the September 25 and the October 17 harvests:

(1) Total length of seminal root axes per plant.

(2) The length of lesions and rotting per millimetre of main root axes.

(3) Total number of leaves per plant at both harvests, and number of tillers for plants sampled on October 17.

(4) Length of the first leaf, except for samples collected on October 17 when the majority of first leaves had begun to senesce.

By October 31 the roots had grown to an extent where it was impossible to remove intact root systems from the soil, so root measurements were not recorded. Leaf measurements were not recorded on this date either. Shoots of all plants were dried and weighed on each of the three sample dates.

Segments of root were removed at random from each set of five plants and plated on RA medium (as described in the General Methods) to determine the species of fungi infecting root tissues. A second set of samples was plated on selective (VP_3) medium to detect infection by *Pythium* spp. (as described in the General Methods). A total of six 1.0 cm root segments for each set of five plants were plated on each medium on September 25, and twelve root segments per set of five plants were plated from samples taken on October 17 and 31.

One seminal root was removed, at random, from each of the five plants on September 25 and October 17, but on October 31 only crown roots were sampled. These seminal or crown roots were stained in lactoglycerol-cotton blue, and examined microscopically to determine numbers of *P. neglectus* and their eggs in root cortices. The number of lateral roots per millimetre of seminal or crown root axes was recorded.

10.3 RESULTS

Symptoms on the Roots

Visual assessment of roots indicated that fumigant tended to enhance root growth and health to a greater extent than did aldicarb, although both chemicals resulted in the production of longer root axes, and a greater root mass overall (Plates 10.1, 10.2). The most striking difference between root systems from areas treated with aldicarb and those from the untreated areas was in the length and number of lateral roots along the main root

axes (Plate 10.2). This was observed on all sample dates. Lateral roots on plants from untreated areas were stunted and rotted, with considerable cortical discolouration. The majority of roots from untreated areas were thus an orange-brown colour. Both chemical treatments resulted in the production of numerous, long, healthy lateral roots (Plates 10.1, 10.2). These laterals (primary laterals) had, in turn, branched to produce secondary laterals.

Most root samples from untreated soil displayed a general discolouration and cortical rotting, rather than the distinct lesions described by Dropkin (1989). However, some root samples collected in September did have distinct orange-brown lesions. These may have been caused by cortical infection with *Pythium* spp., *Fusarium* spp., *Microdochium bolleyi* or *Rhizoctonia solani*, or a mixed infection of these fungi. All these species were subsequently isolated from the roots, with *Fusarium* and *Pythium* spp. dominating the fungal flora (Tables 10.1a - 10.8a). Numerous oospores of *Pythium* spp. were observed in roots stained with lactoglycerol-cotton blue. Hyphae of *R. solani* were seen on the surface of roots in mid-September, prior to the development of severe root rotting. Whether these hyphae had penetrated epidermal or cortical cells was not determined, and these may in fact have only been growing externally on the root surface. Kerr (1955) found that 70% of *R. solani* isolates from inside patches of poor growth were pathogenic, but only 12.5% from outside these patches were pathogenic to wheat. As distinct patches did not occur in the areas sampled in 1989, it is reasonable to assume that the majority of isolates were growing saprophytically on or within roots. *Gaeumannomyces graminis* hyphae were also seen on the surface of some root samples.

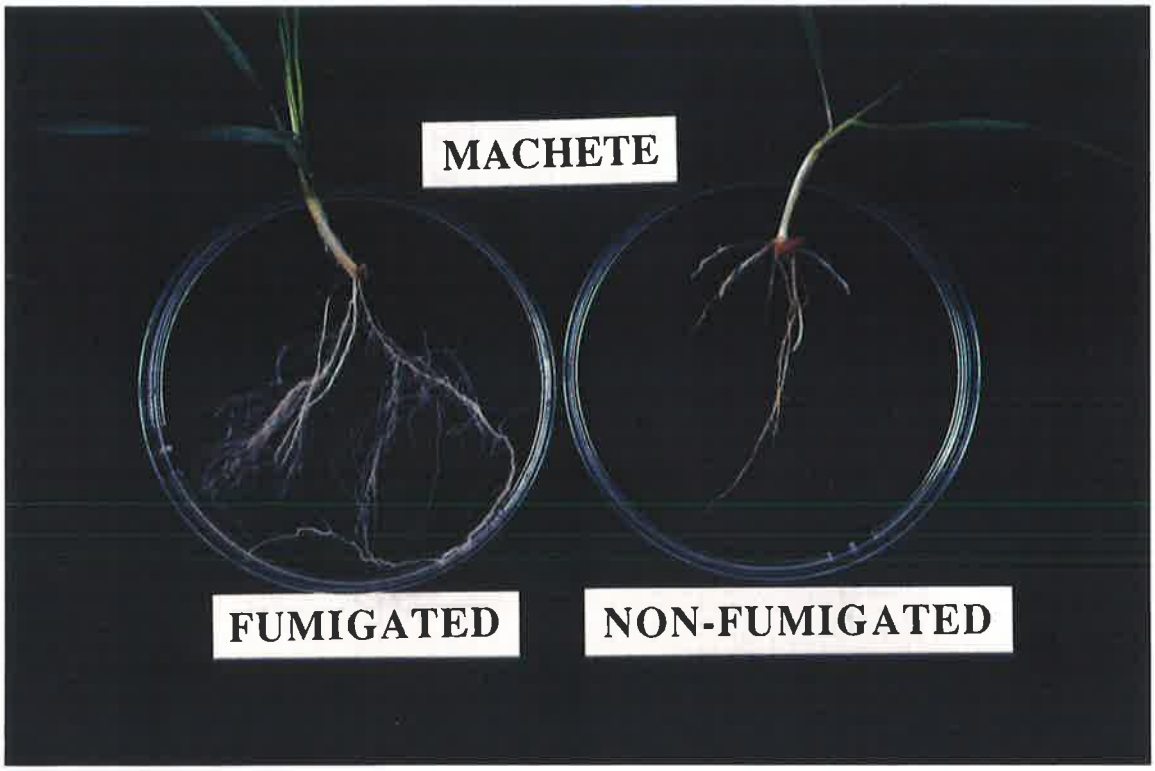
Crown roots were rotted by late October, with lesioned and decorticated segments, as outlined in the previous chapter. These roots sustained damage similar to that observed on seminal roots, but as crown roots develop four to six weeks after seminal roots, symptom development on these roots was delayed until late September-early October. Crown root laterals were damaged in the same manner as that described for seminal root laterals.

Both methyl bromide and aldicarb reduced the length of lesioning and rotting on the main root axes, although the fumigant was more effective in alleviating this root

PLATE 10.1: Roots of Machete (**A**) and Warigal (**B**) wheat sampled from fumigated and non-fumigated areas at Cambrai on September 25, 1989. Fumigation greatly enhanced lateral root growth, and reduced the incidence of lesioning and decortication.

Plate 10.1

A


MACHETE
FUMIGATED
NON-FUMIGATED

B

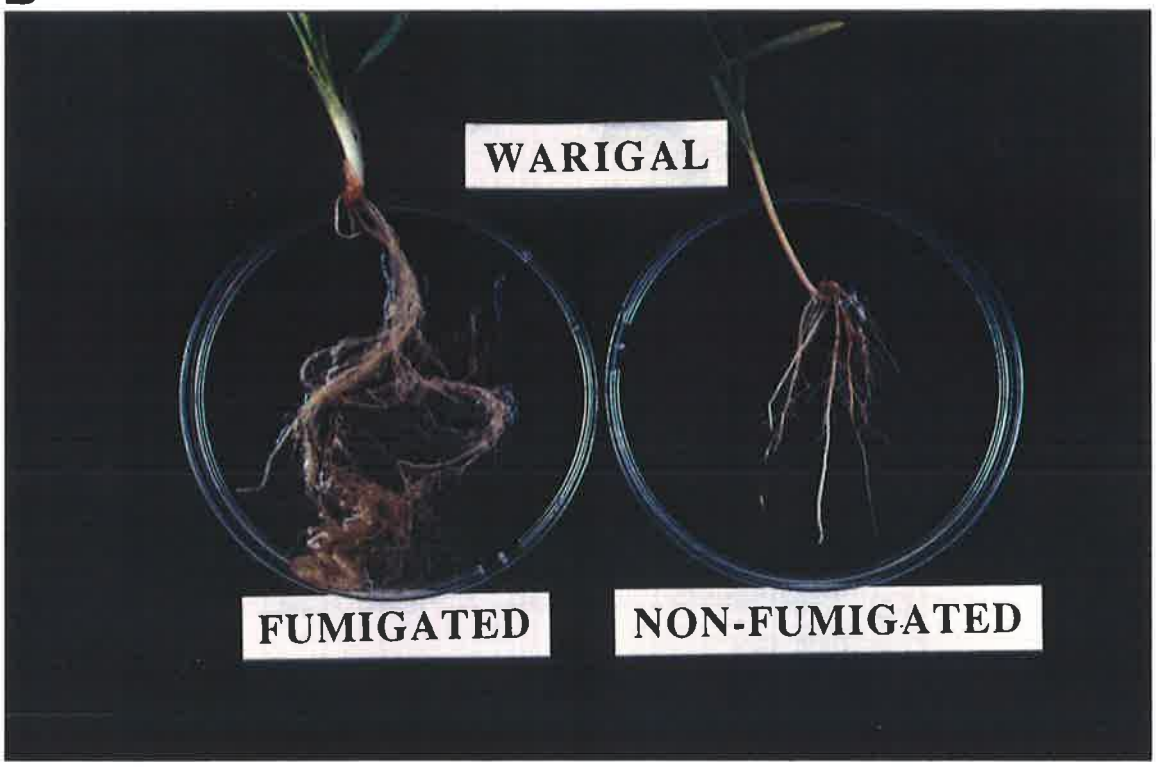
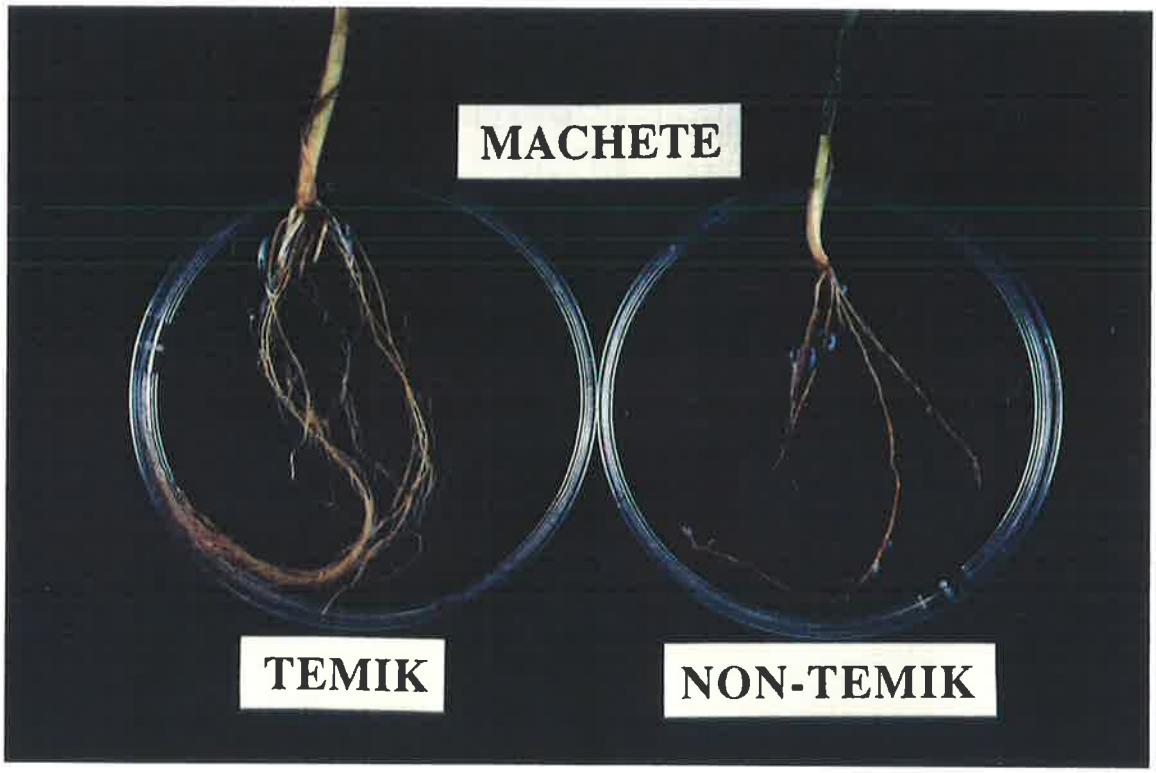

WARIGAL
FUMIGATED
NON-FUMIGATED

PLATE 10.2: Roots of Machete wheat sampled at Cambrai in September, 1989, from areas treated with nematicide (**Temik®**) and from untreated areas (**Non-Temik®**). Roots from the untreated area are severely discoloured, with lateral roots reduced to short "stumps" or "spikes".

Plate 10.2



damage. Aldicarb reduced the extent of lesioning measured in late September by up to 51% (Tables 10.1c, 10.2c), but had little effect in October (Tables 10.3c - 10.8c). Fumigant consistently reduced the extent of lesioning by 90 - 98%, through September and October, but this was only significant ($P<0.05$) for Warigal at Mannum on October 17 (Table 10.3c). Roots from untreated areas at the Cambrai site suffered more lesioning and rotting than those from Mannum, which was reflected in the higher numbers of *P. neglectus* and their eggs in roots from Cambrai (Tables 10.1c - 10.8c).

Fungi Isolated From the Roots

Fusarium spp. (other than *F. graminearum*) - Along with species of *Pythium*, the fusaria were the most frequently encountered fungi. In September and early October, fumigation reduced the frequency of *Fusarium* spp. isolated from roots of both varieties (Tables 10.1a - 10.6a), while aldicarb reduced their occurrence on Machete (Tables 10.5a, 10.6a). In late October, the frequency of fusaria isolated was decreased by both chemical treatments (Tables 10.7a, 10.8a). Frequency of *Fusarium* spp. was not, however, significantly reduced by either treatment on any sample date. There was no difference between sites or varieties in the frequency of *Fusarium* spp. isolated from the roots on untreated areas (Tables 10.1a - 10.8a).

Pythium spp. - These fungi were recorded at high frequencies on all samples, but again the low number of replicates resulted in no statistically significant differences between treatments. Both chemicals reduced the incidence of *Pythium* infecting Warigal roots at Cambrai on September 25 (Table 10.1a). This was also the case for Machete roots at Mannum and Cambrai on October 17 (Table 10.5a) and 31 (Table 10.8a), respectively. Treatment with aldicarb reduced the incidence of *Pythium* spp. isolated from Machete (Table 10.6a) and Warigal (Table 10.4a) roots at Cambrai on October 17. Aldicarb generally reduced *Pythium* infection to a greater degree than did methyl bromide. At Cambrai in both September and October, *Pythium* spp. infected Machete (Tables 10.2a, 10.6a, 10.8a) roots from untreated areas at higher frequencies than they infected Warigal (Tables 10.1a, 10.4a, 10.7a) roots. However, at Mannum on October 17, *Pythium* spp. were isolated more frequently from Warigal (Table 10.3a) than from

Machete (Table 10.5a).

Microdochium bolleyi - The frequency of this fungus in Machete roots at Cambrai was reduced by methyl bromide and aldicarb on September 25 (Table 10.2a), but this was not statistically significant. Both treatments significantly ($P<0.05$) reduced the frequency of *M. bolleyi* in Warigal roots at Cambrai on October 17 (Table 10.4a). Aldicarb also reduced the frequency of *M. bolleyi* isolated from Machete roots at Cambrai on October 17 (Table 10.6a) and 31 (Table 10.8a). *M. bolleyi* was isolated more often from untreated areas at Cambrai than at Mannum. This species was more frequent on Machete (Table 10.2a) than on Warigal (Table 10.1a) in September, but the reverse was true on October 17 (Tables 10.3a - 10.6a).

Other Species - *Rhizoctonia solani*, *Bipolaris sorokiniana* and *Gaeumannomyces graminis* were isolated sporadically and at low frequencies, while *F. graminearum* was not isolated from the roots of any plants sampled. *G. graminis* was not isolated from roots in September (Tables 10.1a, 10.2a), and there were only two instances in October when this species was recorded from 4% of roots from untreated areas (Tables 10.3a, 10.6a). Furthermore, symptoms of *G. graminis* infection (distinct, black, stelar lesions and blackening of stem bases) were not apparent. *R. solani* infected 0 - 17% of roots from untreated areas, with higher frequencies recorded at Mannum (Tables 10.3a, 10.5a) than at Cambrai (Tables 10.4a, 10.6a) on October 17. *B. sorokiniana* infected 0 - 25% of these roots. This value of 25% was recorded at the first sample date in September (Table 10.1a), but in October all infection levels were below 13% (Tables 10.3a - 10.8a). *B. sorokiniana* primarily infects subcrown internode tissues, which were not investigated in this experiment.

Chemical treatments had no consistent effect on the frequency of *R. solani* infecting roots, and none of the differences between treatments were significant. The same was generally true for *B. sorokiniana*, although aldicarb and methyl bromide did significantly ($P<0.05$) reduce the incidence of this species in Warigal roots at Cambrai on September 25 (Table 10.1a). In view of the infrequent isolation of *R. solani*, *B. sorokiniana* and *G. graminis*, and the ineffectiveness of chemical treatments in reducing their occurrence in roots, they did not contribute significantly to the observed root

damage.

Nematode Numbers in Roots

Methyl bromide and aldicarb were equally effective in eliminating *Pratylenchus neglectus* and their eggs from root cortices. Reductions in nematode numbers at Cambrai on October 17 were significant ($P<0.05$) for both Machete (Table 10.6c) and Warigal (Table 10.4c) treated with either chemical. At Cambrai on October 17, *P. neglectus* eggs were absent from Machete roots (Table 10.6c), and this was a significant reduction ($P<0.05$) for both chemical treatments. The effect of fumigation and aldicarb in freeing roots of *P. neglectus* and their eggs persisted through September and October. Machete roots (Tables 10.2c, 10.5c, 10.6c, 10.8c) from untreated areas were infected with more *P. neglectus* than were Warigal roots (Tables 10.1c, 10.3c, 10.4c, 10.7c), and higher nematode numbers were recorded at Cambrai (Tables 10.1c, 10.2c, 10.4c, 10.6c - 10.8c) than at the Mannum site (Tables 10.3c, 10.5c). Numbers of *P. neglectus* eggs within roots followed the same pattern.

Plant Growth

Differences in plant growth between treatments are demonstrated in Plate 10.3. Fumigation consistently enhanced the length of seminal root axes. However, this was only significant ($P<0.05$) for Warigal at Cambrai on September 25 (Table 10.1b), when seedlings from areas treated with methyl bromide produced roots 33% longer than on the untreated area. By October 17, fumigation had enhanced, non-significantly, the length of Warigal roots by 11 - 25% (Tables 10.3b, 10.4b) and that of Machete roots by only 1 - 6% (Tables 10.5b, 10.6b). The effect of methyl bromide on root length was generally less in October (Tables 10.3b - 10.8b) than in September (Tables 10.1b, 10.2b).

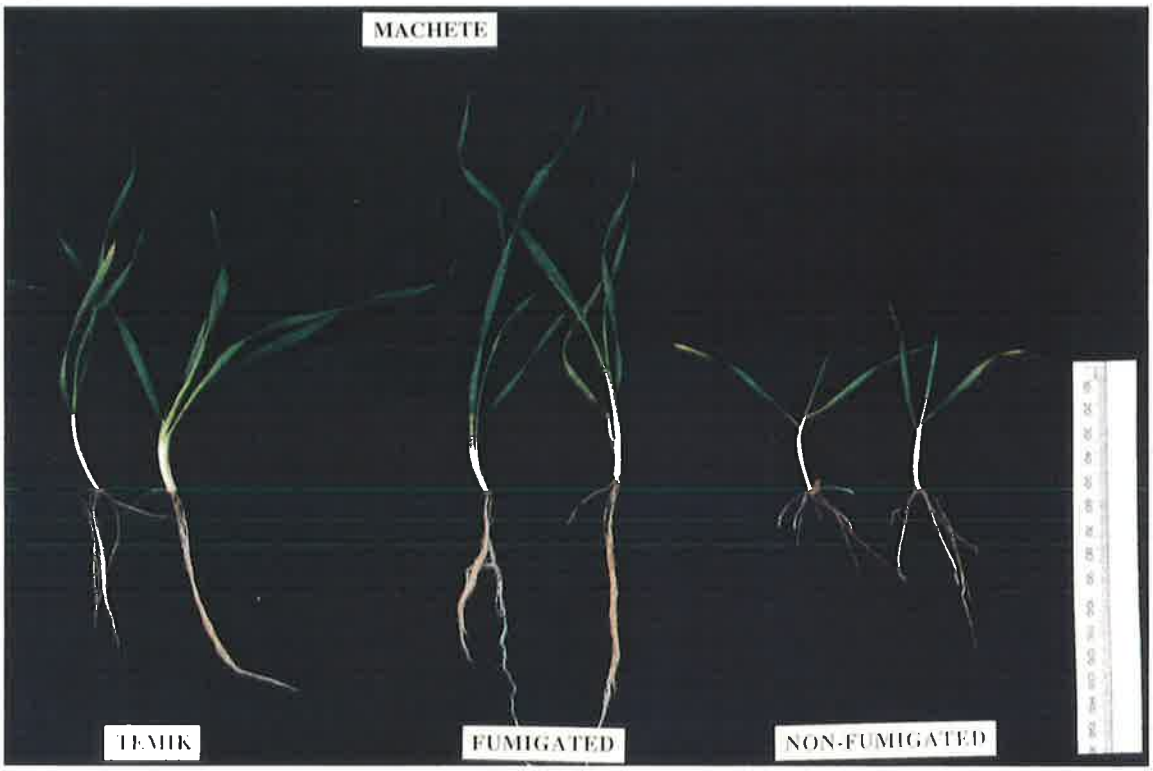
Aldicarb treatment led to a non-significant 8% increase in the growth of Warigal roots at Cambrai on October 17 (Table 10.4b), but at no other time was there a measurable increase in root length due to the nematicide. In fact, aldicarb reduced the extent of root growth in some instances (Tables 10.2b, 10.3b. 10.5b, 10.6b).

At Cambrai in September, both varieties had similar root lengths on untreated

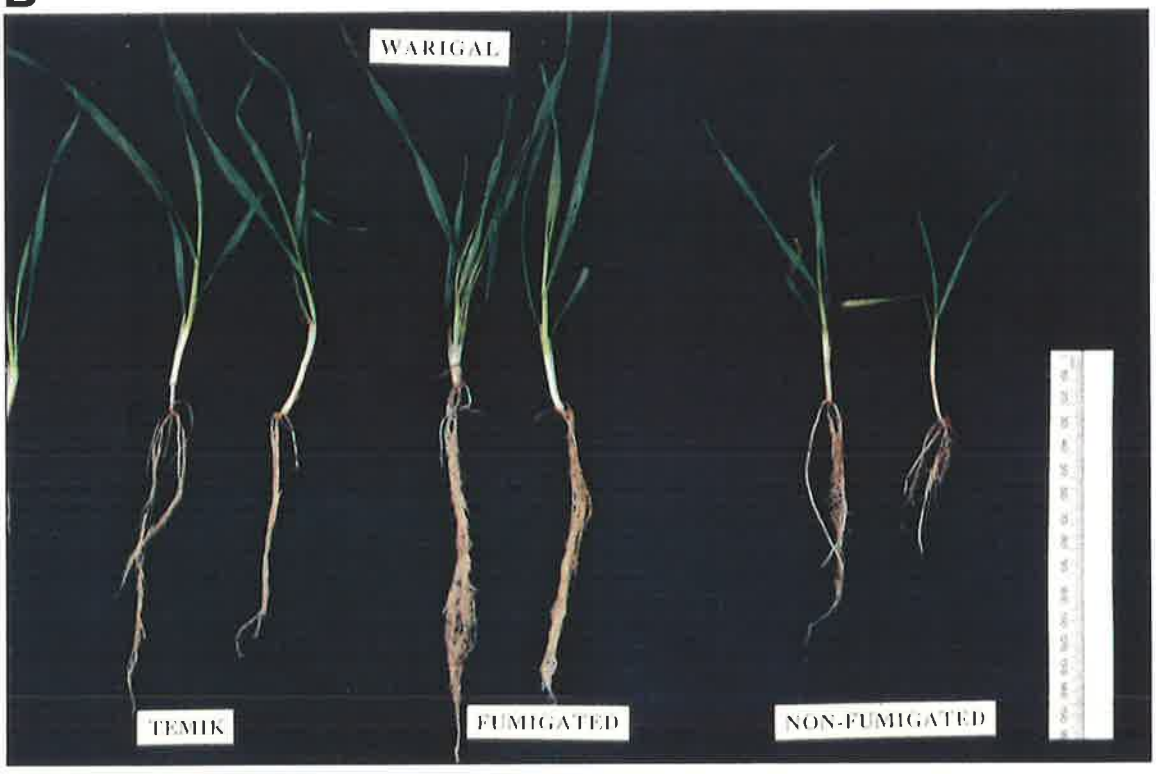
PLATE 10.3: Machete (**A**) and Warigal (**B**) wheat sampled from Cambrai on September 25, 1989. Plants were sown on August 25 on areas treated with nematicide (**Temik®**), methyl bromide (**Fumigated**) or on sections that were untreated (**Non-Fumigated**). Both chemicals enhanced root and shoot growth, although fumigant improved growth to a greater extent than did nematicide.

Plate 10.3

A


MACHETE
TEMIK
FUMIGATED
NON-FUMIGATED

B


WARIGAL
TEMIK
FUMIGATED
NON-FUMIGATED

areas (Tables 10.1b, 10.2b). On October 17, Machete roots (Table 10.6b) were longer than those of Warigal (Table 10.4b) at Cambrai, but the reverse was true at Mannum (Tables 10.3b, 10.5b).

On most sample dates, the number of leaves on both Warigal and Machete seedlings was not appreciably affected by either chemical at either site. However, at Mannum on October 17, fumigation increased the total leaf number on Warigal by 47% (Table 10.3b), but this large increase is misleading, as it was not statistically significant, and reflected the extra tillering of these plants. Methyl bromide generally had a greater stimulatory effect on leaf numbers than did aldicarb.

Both chemical treatments increased tillering of Machete (Table 10.5b) and Warigal (Table 10.3b) at Mannum on October 17, although not significantly. Fumigation led to 54% and 13% more tillers on Warigal and Machete plants, respectively. Aldicarb resulted in Warigal and Machete producing 27% and 24% more tillers, respectively. Neither treatment enhanced tillering at Cambrai, and the number of tillers was in fact reduced by aldicarb (Tables 10.4b, 10.6b).

The length of the first leaf, measured on September 25, was increased by both chemicals, with a tendency for aldicarb to enhance leaf length to a greater extent than did the fumigant. Warigal leaves were 12% longer after fumigation and 22% longer after aldicarb treatment (Table 10.1b). Leaves on Machete seedlings were 14% longer due to fumigation and 30% longer due to aldicarb treatment (Table 10.2b). Leaf length, however, was not significantly enhanced by application of either chemical.

Aldicarb and methyl bromide improved shoot growth on September 25 (Tables 10.1b, 10.2b), whereas only methyl bromide increased this parameter on October 17 (Tables 10.3b - 10.6b). On September 25, methyl bromide increased shoot dry weight by 19 - 42%. Aldicarb did not improve shoot growth in October, but methyl bromide treatment resulted in an increase in shoot weight of 13 - 65%. However, shoot growth was not enhanced significantly by either treatment. Neither treatment increased shoot growth of samples taken on October 31 (Tables 10.7b, 10.8b). Aldicarb enhanced shoot growth measured in September, whereas the effect of fumigation persisted to mid-October.

Both varieties had similar shoot weights on untreated areas at Cambrai in September (Tables 10.1b, 10.2b) and on October 17 (Tables 10.3b - 10.6b). However, shoots of Warigal (Table 10.7b) weighed more than those of Machete (Table 10.8b) at Cambrai on October 31. On October 17, root length and shoot weight were greater at Cambrai (Tables 10.4b, 10.6b) than at Mannum (Tables 10.3b, 10.5b).

Treatment with aldicarb significantly ($P<0.05$) enhanced the production of lateral roots on Machete seedlings at Cambrai on September 25 (Table 10.2c), when this treatment led to the production of 33% more laterals. On October 17, Warigal roots at Mannum (Table 10.3c) produced 53% more laterals due to fumigation, which was also significant ($P<0.05$). Fumigant seemed to be more effective and increased lateral root numbers by up to 53%, while aldicarb increased this variable up to 33%. Aldicarb improved lateral root growth in September (Tables 10.1c, 10.2c), but thereafter its influence on lateral roots diminished (Tables 10.3c - 10.8c). The effect of fumigation tended to persist into October. Machete (Table 10.2c) and Warigal (Table 10.1c) on untreated areas at Cambrai produced similar numbers of laterals in September, but Warigal (Tables 10.3c, 10.4c, 10.7c) roots had more laterals than Machete (Tables 10.5c, 10.6c, 10.8c) roots in October. Warigal roots also contained fewer *P. neglectus* and their eggs in October.

TABLES 10.1 - 10.8: The effect of methyl bromide soil fumigant and nematicide (aldicarb) on the frequency (% root segments infected) of fungi isolated from wheat roots, on shoot and root growth, and on numbers of *Pratylenchus neglectus* and their eggs in root cortices. (Values are the mean of two replicates for each treatment). Values in brackets for fungal frequencies are arcsine √% transformed means, and the LSD (0.05) is based on these transformations.

TABLE 10.1: Warigal wheat sampled at Cambrai on September 25, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
***Fusarium* spp.**	33.3	(34.6)	91.7	(82.2)	100.0	(98.5)	50.3
F. graminearum	0	(1.5)	0	(1.5)	0	(1.5)	-
M. bolleyi	8.3	(12.8)	8.3	(12.8)	25.0	(29.7)	20.2
R. solani	0	(1.5)	8.3	(12.8)	0	(1.5)	39.7
B. sorokiniana	0	(1.5)	25.0	(29.7)	0	(1.5)	19.5
G. graminis	0	(1.5)	0	(1.5)	0	(1.5)	-
***Pythium* spp.**	83.3	(76.6)	100.0	(98.5)	66.7	(54.8)	76.8
(b) PLANT GROWTH							
Leaves/Plant	4.4		3.7		4.0		0.9
1st Leaf (mm)	77.9		68.5		87.3		76.8
Tillers/Plant	...		...		...		...
Root Length (mm)	418.0		278.9		278.7		82.6
Shoot Wt.(mg/plant)	47.0		27.6		47.8		59.1
(c) ROOTS							
Lesions/mm of Root	0.01		0.12		0.06		0.12
Nematodes/mm	...		0.35		...		...
Eggs/mm of Root	...		0.09		...		...
Laterals/mm of Root	...		0.17		...		...

TABLE 10.2: Machete wheat sampled at Cambrai on September 25, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
***Fusarium* spp.**	41.7	(40.1)	91.7	(82.2)	91.7	(82.2)	100.7
F. graminearum	0	(1.5)	0	(1.5)	0	(1.5)	-
M. bolleyi	25.0	(29.7)	41.7	(39.4)	16.7	(24.1)	47.2
R. solani	25.0	(23.3)	0	(1.5)	16.7	(24.1)	76.4
B. sorokiniana	0	(1.5)	16.7	(24.1)	0	(1.5)	-
G. graminis	0	(1.5)	0	(1.5)	0	(1.5)	-
***Pythium* spp.**	83.3	(76.6)	41.7	(39.4)	91.7	(82.2)	21.5
(b) PLANT GROWTH							
Leaves/Plant	3.4		3.7		3.3		1.0
1st Leaf (mm)	66.5		57.2		82.2		35.1
Tillers/Plant	...		...		...		...
Root Length (mm)	345.1		269.9		240.5		218.3
Shoot Wt.(mg/plant)	31.8		28.8		35.5		33.3
(c) ROOTS							
Lesions/mm of Root	0.01		0.13		0.10		0.27
Nematodes/mm	...		0.57		0		2.17
Eggs/mm of Root	...		0.11		0		1.01
Laterals/mm of Root	...		0.18		0.27		0.03

TABLE 10.3: Warigal wheat sampled at Mannum on October 17, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
***Fusarium* spp.**	70.8	(57.8)	100.0	(97.0)	91.7	(81.4)	47.4
F. graminearum	0	(3.0)	0	(3.0)	0	(3.0)	-
M. bolleyi	54.2	(47.9)	12.5	(20.4)	20.8	(27.1)	74.7
R. solani	0	(3.0)	4.2	(9.9)	4.2	(9.9)	24.1
B. sorokiniana	0	(3.0)	0	(3.0)	8.3	(13.6)	37.1
G. graminis	0	(3.0)	4.2	(9.9)	0	(3.0)	24.1
***Pythium* spp.**	79.3	(63.0)	70.8	(59.1)	75.0	(61.5)	55.9
(b) PLANT GROWTH							
Leaves/Plant	13.7		7.2		7.1		10.9
1st Leaf (mm)	...		...		...		...
Tillers/Plant	3.5		1.6		2.2		2.1
Root Length (mm)	365.4		274.5		265.0		150.6
Shoot Wt.(mg/plant)	279.4		96.4		99.0		248.1
(c) ROOTS							
Lesions/mm of Root	0.01		0.24		0.26		0.01
Nematodes/mm	0		0		0		0.01
Eggs/mm of Root	0		0		0		-
Laterals/mm of Root	0.76		0.36		0.41		0.17

TABLE 10.4: Warigal wheat sampled at Cambrai on October 17, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
***Fusarium* spp.**	91.7	(81.4)	95.8	(85.1)	95.8	(85.1)	13.0
F. graminearum	0	(3.0)	0	(3.0)	0	(3.0)	-
M. bolleyi	4.2	(9.9)	20.8	(26.0)	4.2	(9.9)	8.4
R. solani	8.3	(13.6)	16.7	(24.1)	8.3	(16.7)	37.1
B. sorokiniana	0	(3.0)	4.2	(9.9)	8.3	(13.6)	32.6
G. graminis	0	(3.0)	0	(3.0)	0	(3.0)	-
***Pythium* spp.**	83.3	(66.6)	70.8	(59.1)	25.0	(28.5)	81.0
(b) PLANT GROWTH							
Leaves/Plant	8.3		9.9		6.6		5.6
1st Leaf (mm)	...		...		...		...
Tillers/Plant	1.9		2.2		1.2		1.3
Root Length (mm)	362.7		321.5		347.7		294.7
Shoot Wt.(mg/plant)	219.8		175.1		165.8		76.1
(c) ROOTS							
Lesions/mm of Root	0.01		0.27		0.24		0.45
Nematodes/mm	0		0.07		0		0.04
Eggs/mm of Root	0		0.03		0		0.09
Laterals/mm of Root	0.59		0.48		0.38		0.78

TABLE 10.5: Machete wheat sampled at Mannum on October 17, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
Fusarium spp.	83.3	(66.6)	95.8	(85.1)	79.2	(62.9)	59.6
F. graminearum	0	(3.0)	0	(3.0)	0	(3.0)	-
M. bolleyi	20.8	(27.1)	4.2	(9.9)	16.7	(24.1)	21.0
R. solani	20.8	(27.1)	0	(3.0)	8.3	(13.6)	43.2
B. sorokiniana	0	(3.0)	12.5	(20.4)	8.3	(16.7)	13.0
G. graminis	0	(3.0)	0	(3.0)	0	(3.0)	-
Pythium spp.	70.8	(59.1)	100.0	(97.0)	87.5	(78.5)	99.6
(b) PLANT GROWTH							
Leaves/Plant	6.6		6.4		7.0		2.0
1st Leaf (mm)	...		...		...		...
Tillers/Plant	1.6		1.4		1.8		1.3
Root Length (mm)	272.1		256.9		195.8		121.0
Shoot Wt.(mg/plant)	121.4		105.1		86.0		95.2
(c) ROOTS							
Lesions/mm of Root	0.01		0.18		0.29		0.21
Nematodes/mm	0.01		0		0.01		0.04
Eggs/mm of Root	0.01		0		0		0.02
Laterals/mm of Root	0.30		0.49		0.30		0.25

TABLE 10.6: Machete wheat sampled at Cambrai on October 17, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
Fusarium spp.	66.7	(56.7)	91.7	(73.3)	83.3	(66.6)	72.5
F. graminearum	0	(3.0)	0	(3.0)	0	(3.0)	-
M. bolleyi	29.2	(30.9)	16.7	(23.4)	8.3	(13.6)	80.7
R. solani	0	(3.0)	0	(3.0)	0	(3.0)	-
B. sorokiniana	4.2	(9.9)	4.2	(9.9)	4.2	(9.9)	48.3
G. graminis	0	(3.0)	4.2	(9.9)	0	(3.0)	24.1
Pythium spp.	79.2	(62.9)	70.8	(59.1)	30.3	(33.4)	53.6
(b) PLANT GROWTH							
Leaves/Plant	7.5		9.4		5.8		17.0
1st Leaf (mm)	...		...		...		...
Tillers/Plant	1.7		2.1		1.2		4.3
Root Length (mm)	358.2		353.1		261.0		574.8
Shoot Wt.(mg/plant)	167.7		176.2		118.5		358.3
(c) ROOTS							
Lesions/mm of Root	0.01		0.31		0.38		0.76
Nematodes/mm	0		0.08		0		0.07
Eggs/mm of Root	0		0.05		0		0.02
Laterals/mm of Root	0.30		0.24		0.22		0.27

TABLE 10.7: Warigal wheat sampled at Cambrai on October 31, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
Fusarium spp.	91.7	(73.3)	95.8	(85.1)	58.3	(51.6)	103.5
F. graminearum	0	(3.0)	0	(3.0)	0	(3.0)	-
M. bolleyi	4.2	(9.9)	0	(3.0)	0	(3.0)	24.1
R. solani	8.3	(13.7)	12.5	(20.4)	58.3	(60.6)	146.6
B. sorokiniana	0	(3.0)	0	(3.0)	4.2	(9.9)	24.1
G. graminis	0	(3.0)	0	(3.0)	0	(3.0)	-
Pythium spp.	87.5	(78.5)	91.7	(81.4)	100.0	(97.0)	103.8
(b) PLANT GROWTH							
Leaves/Plant	...		...		...		...
1st Leaf (mm)	...		...		...		...
Tillers/Plant	...		...		...		...
Root Length (mm)	...		...		...		...
Shoot Wt.(mg/plant)	547.4		694.0		467.8		1002.8
(c) ROOTS							
Lesions/mm of Root	...		...		...		...
Nematodes/mm	0		0.01		0		0.03
Eggs/mm of Root	...		...		...		...
Laterals/mm of Root	0.12		0.13		0.14		0.35

TABLE 10.8: Machete wheat sampled at Cambrai on October 31, 1989.

	Fumigated		Untreated		Aldicarb		LSD (0.05)
(a) FUNGI (% infection)							
***Fusarium* spp.**	95.0	(84.3)	95.8	(85.1)	66.7	(54.8)	74.8
F. graminearum	0	(3.0)	0	(3.0)	0	(3.0)	-
M. bolleyi	15.0	(18.1)	4.2	(9.9)	0	(3.0)	46.0
R. solani	5.0	(10.7)	0	(3.0)	30.6	(33.0)	29.3
B. sorokiniana	0	(3.0)	8.3	(16.7)	11.1	(15.6)	44.1
G. graminis	0	(3.0)	0	(3.0)	0	(3.0)	-
***Pythium* spp.**	85.7	(77.3)	87.5	(78.5)	66.7	(66.1)	160.3
(b) PLANT GROWTH							
Leaves/Plant	...		...		...		...
1st Leaf (mm)	...		...		...		...
Tillers/Plant	...		...		...		...
Root Length (mm)	...		...		...		...
Shoot Wt.(mg/plant)	359.9		539.4		415.7		768.6
(c) ROOTS							
Lesions/mm of Root	...		...		...		...
Nematodes/mm	0		0.05		0.01		0.17
Eggs/mm of Root	...		...		...		...
Laterals/mm of Root	0.03		0.07		0.05		0.18

10.3.1 Summary of Results

Treatment of field sites in the Murray Mallee with nematicide (aldicarb) or soil fumigant (methyl bromide) in August, 1989 demonstrated that:

(1) Both chemical treatments significantly enhanced the production of lateral roots along main root axes.

(2) Root health was improved more by methyl bromide than by aldicarb, with the extent of lesioning and rotting on main root axes being reduced. Root length was consistently enhanced by methyl bromide, but aldicarb was less effective in improving this growth parameter.

(3) Methyl bromide and aldicarb were equally effective in eradicating *P. neglectus* and

their eggs from root cortices, and this effect persisted through September and October.

(4) Leaf and tiller numbers were increased more by methyl bromide than by aldicarb treatment, whereas aldicarb had a greater impact on the leaf length. However, these differences were not statistically significant.

(5) Aldicarb and methyl bromide enhanced shoot growth in September, although not significantly. The effect of methyl bromide persisted until mid-October. Neither chemical had improved shoot growth measured in late October.

(6) Fungal infection of roots was reduced by fumigation, but the nematicide also influenced the frequency of fungi isolated. *Pythium* and *Fusarium* spp. were the most commonly encountered fungi, while *F. graminearum* was absent and *G. graminis* extremely rare.

10.4 DISCUSSION

Both chemicals were equally effective in excluding *P. neglectus* and their eggs from root cortices. As nematodes were eradicated, or drastically reduced in number, enhanced plant growth caused by aldicarb treatment can be attributed to the removal of *P. neglectus* (although fungi have some interactive role), and the greater improvement in plant growth due to fumigation is due to the combined effect of removing *P. neglectus* and reducing fungal infection of roots. In northern New South Wales, Doyle *et al.* (1987) found that methyl bromide controlled *P. thornei* and fungi in wheat crops, resulting in a yield increase of 78%. Aldicarb controlled *P. thornei*, without influencing fungi, increasing yield to a lesser degree than that achieved with fumigation (Doyle *et al.*, 1987). The results of chemical treatment at Murray Mallee field sites in 1989 suggest that *P. neglectus* is at least partly responsible for damage to wheat roots, consequently inhibiting root and shoot growth, but fungi are intimately involved with this nematode problem.

Although aldicarb and methyl bromide considerably reduced numbers of *P. neglectus* and their eggs in roots, few of these differences were statistically significant. Data are based on only two replicates, and these varied considerably. *Pratylenchus* spp.

tend to congregate in roots, as they are attracted to sites that have already been invaded (Baxter and Blake, 1968; Corbett, 1972; Zunke, 1990). This leads to the phenomenon of many nematodes occurring in groups at intervals along the root (Baxter and Blake, 1968), and some roots, especially those that have suffered extensive cortical damage, contain few or no nematodes. As root invasion by *P. neglectus* does not occur at random, it is difficult to accurately assess infection levels by taking random samples from the field. Increased replication would partly alleviate this problem, although better methods of sampling may also be required. Enumeration of nematodes in the whole root system, plus surrounding soil, is also desirable. Nevertheless, this experiment indicated the effect of these nematodes on the growth of wheat, as well as demonstrating the possible involvement of fungi in a disease complex.

Fumigation decreased fungal infection of roots, as expected, but the nematicide also reduced the occurrence of fungi within wheat roots. *Pythium* spp. (Tables 10.1a, 10.4a, 10.5a, 10.6a, 10.8a), *Fusarium* spp. (other than *F. graminearum*) (Tables 10.3a, 10.5a - 10.8a) and, to some extent, *Rhizoctonia solani* (Tables 10.1a, 10.4a) and *Microdochium bolleyi* (Tables 10.2a, 10.4a, 10.6a, 10.8a), were reduced in frequency by aldicarb treatment. Aldicarb can directly inhibit the growth of some fungi. Tisserat *et al.* (1977) reported that, *in vitro*, aldicarb inhibited mycelial growth of *R. solani*. Conversely, the incidence of *R. solani* damping-off of sugar beet seedlings increased after the addition of aldicarb to soil inoculated with the fungus (Tisserat *et al.*, 1977), and on October 31 high levels of *R. solani* were detected in the areas that had been treated with aldicarb (Tables 10.7, 10.8). However, aldicarb did not significantly increase fungal infection of wheat roots, and in many cases led to reduced levels of infection. This may suggest that the nematicide reduces fungal infection by excluding *P. neglectus* from the roots. Tissues already invaded by nematodes would be readily infected by fungi, and consequently suffer more extensive damage. Cortical lesions caused by *Pratylenchus* spp. are excellent sites for invasion of roots by other pathogens (Dropkin, 1989).

This phenomenon has been documented since 1892, when Atkinson (1892) found that *Fusarium* wilt of cotton was exacerbated by concomitant infection of roots with root knot nematodes (*Meloidogyne*). Since then, many workers have investigated the

synergistic, or at least close, associations between fungi and nematodes in causing root disease. However, the mechanisms whereby these interactions occur are difficult to define, and different mechanisms probably apply to individual host-nematode-fungus combinations (Riedel, 1988).

The mechanisms postulated fall into three categories: vectors, ingress and predisposition (Riedel, 1988). Nematodes may act as vectors for viruses, and perhaps carry soil-borne bacteria. This does not usually apply to soil-borne fungi, although Kurpa and Vrain (1989) have detected *Gnomonia comari* spores attached to the cuticle of *P. penetrans* associated with strawberry plants. *Pratylenchus* spp. are not among the four genera of phytophagous nematodes known to transmit plant viruses (Riedel, 1988), nor can stylet-bearing nematodes ingest bacteria. *P. neglectus* is therefore unlikely to act directly as a vector enabling ingress of bacteria or fungi into roots under the circumstances investigated here.

Wounding of root tissues by nematodes is undoubtedly an important factor enhancing ingress of fungi, although many interactions cannot be explained in terms of wounding alone, and experimental wounding of tissues often has no effect on the extent of fungal infection (Riedel, 1988). When peppermint plants were grown with split root systems and these individually inoculated with *P. neglectus* or *Verticillium dahliae*, a synergistic reaction still occurred, although the pathogens were restricted to different parts of the same root system (Faulkner and Skotland, 1965; Faulkner *et al.*, 1970). This suggests that the nematode induces changes in the host tissues, making the roots more attractive or less tolerant to fungal infection (Faulkner *et al.*, 1970). However, similar experiments with *V. dahliae* and *P. penetrans* on tomato were unsuccessful, suggesting that wounding is the mechanism at work in this interaction (Conroy *et al.*, 1972). The fact that root wounding is not the sole reason for increased fungal infection in the presence of nematodes is demonstrated on potatoes. Three species of *Pratylenchus* (*P. penetrans*, *P. crenatus* and *P. scribneri*) readily feed and reproduce within potato roots, but only *P. penetrans* elicits a strong synergistic interaction with *V. dahliae* (Riedel and Rowe, 1985).

Fungi are usually capable of infecting root tissues in the absence of other microorganisms, so nematodes are a modifying rather than essential factor in most

interactions. Nematodes can predispose plants to elevated infection levels or more severe damage by fungi due to physiological or biochemical changes brought about in the host. This is most dramatic when nematodes render plants susceptible to a fungus to which these plants are otherwise resistant. For example, *P. penetrans* can break down resistance in pea to *Fusarium oxysporum* f. sp. *pisi* (Oyekan and Mitchell, 1971).

Nematode infection can cause stress to the host by limiting root development (Riedel, 1988), thus rendering plants more susceptible to fungal infection. Unlike most nematodes, *Pratylenchus* spp. reproduce more actively in a stressed host (Dropkin, 1989). Orion *et al.* (1984) found that *P. mediterraneus* (which they had originally described as *P. thornei*) in Israel occurred at higher populations and caused more damage to wheat roots when water was a limiting factor. Nematodes also cause increased levels of root exudation and cell sloughing, in turn bringing about changes in the rhizosphere microflora which can alter the course of disease in the host. Nematodes can even predispose plants to damage by fungi that are not usually considered pathogenic. In India, Nath and Kamalwanshi (1989) found that *Aspergillus niger*, *Rhizopus nigricans*, *Penicillium digitatum*, *Curvularia trifolii* or *Rhizoctonia bataticola* alone caused no necrosis of tomato seedling roots, but all produced root necrosis when *Meloidogyne javanica* was also inoculated onto plants.

With so many species of fungi infecting wheat roots in the field, numerous possibilities exist for interactions with the nematode population within root tissues. The mechanisms involved may include a combination of those discussed above. At least one, and possibly several, of the fungal species investigated are probably affected by the presence of *P. neglectus*. The reverse is unlikely (namely, that fungal infection increases nematode populations in the roots) for several reasons. *Pratylenchus* spp. are not likely to infect roots already rotted by fungi, and are known to vacate extensively damaged tissues in search of a fresh food source (Corbett, 1972; Dropkin, 1989), whether this damage is due to the nematodes themselves, or brought about by fungal infection. Reports indicate that numbers of *Pratylenchus* spp. in roots decline with high levels of fungal infection (Bhatt, 1986), and this is also true of other nematodes (Labuschagne *et al.*, 1989; Chandel and Sharma, 1989), especially sedentary endoparasites like *Meloidogyne* and

Heterodera (Patel, 1983; Walia and Gupta, 1986; Starr *et al.*, 1989).

Of the fungi that may have been involved with *P. neglectus* in the field in 1989, *Fusarium* spp., *Pythium* spp. and *R. solani* have been recorded in association with *Pratylenchus* spp., and other nematodes, often acting synergistically (Benedict and Mountain, 1956; Mauza and Webster, 1982; Townshend, 1984). *M. bolleyi*, however, has never been investigated in relation to nematode infection, nor has this fungus been thoroughly studied on cereals.

R. solani and *P. neglectus* have been found in association with patches of poor growth in South Australian cereal crops (de Beer, 1965), where a synergistic relationship was postulated, but not proven. These two pathogens do react synergistically on wheat in Ontario, Canada (Benedict and Mountain, 1956), where the combined effects of fungus and nematode are twice that of either pathogen by itself. High levels of *P. penetrans* increase the severity of *R. fragariae* root rot of strawberry (LaMondia and Martin, 1988, 1989). Combined infections of *M. javanica* and *R. bataticola* increase damage to chickpea in India (Goel and Gupta, 1986), but there is no significant interaction between this fungus and *Heterodera cajani* on cowpea, although these occur together on plants in the field (Walia and Gupta, 1986).

Meagher and Chambers (1971) reported that *R. solani* and *H. avenae* acted synergistically on wheat in glasshouse studies in Australia. However, this is unlikely to occur in the field, as sedentary endoparasitic nematodes (like *H. avenae*) would be adversely affected by fungal damage to syncytial and surrounding cells, and the nematode would invariably die as a result. Unlike *Pratylenchus* spp., cyst-forming nematodes cannot migrate to undamaged tissues to continue feeding. Patel (1983), in fact, demonstrated that when *R. solani* infection preceded inoculation with *H. avenae*, roots were made unsuitable for the nematode.

Disease caused by *Fusarium* spp. is often exacerbated in the presence of *Pratylenchus* spp. and other nematodes. *P. penetrans* alone causes root disease of lucerne (alfalfa; *Medicago sativa*), but in conjunction with *F. oxysporum* the degree of lesioning to roots and stunting of plants is increased (Mauza and Webster, 1982). *Tylenchulus semipenetrans* and *F. solani* both injure citrus roots in India, but the fungus is more

damaging when the nematode is also present (Chandel and Sharma, 1989). Labuschagne *et al.* (1989) also found that there was a significant interaction between *F. solani* and *T. semipenetrans* on citrus roots.

Species of *Pythium* are implicated in these relationships with nematodes, although less frequently than the species of fungi mentioned above. Rhizome rot of ginger in India is a complex disease, where infection with rhizome fly (*Mimegralla coeruleifrons*) or with nematodes (*M. incognita*, *P. coffeae*) predisposes plants to infection by fungi, including *Pythium pleroticum* and *P. aphanidermatum* (Dohroo *et al.*, 1987). Townshend (1984) suggested that a fungus-nematode interaction might be involved in reducing lucerne stands, as *M. hapla* and *Pythium ultimum* reduce stands, whereas the nematode by itself does not affect seedling establishment. However, in South Australia, Singh (1984) and Bratoloveanu (1985) found no evidence for a significant interaction between *P. thornei* and *Pythium irregulare* in root rot of barley or wheat. The effect of a *Pythium* sp. and *P. neglectus* on root rot of wheat, alone and in combination, will be more fully discussed in the following chapter.

Besides these specific relationships between nematodes and fungi, there are also reports implicating *Pratylenchus* spp. in conjunction with a range of fungal pathogens, in what may be termed disease complexes. In the Murray Mallee of South Australia, Patel (1983) detected *P. neglectus* as well as *H. avenae*, *R. solani* and *G. graminis* within patches of poor growth in cereal crops. She concluded that these organisms all contributed to crop damage, although there was an inconsistent relationship between *P. neglectus* and growth variables. Stynes (1975) studied the same suite of organisms on the Yorke Peninsula of South Australia, with similar results to those of Patel (1983). He concluded, on the basis of correlative relationships, that, although ubiquitous in wheat crops, *P. neglectus* only retarded plant growth early in the growing season, but the extent of *P. neglectus* infection was not related to final crop yield. The fungi increased in importance as the season progressed, and the frequency of fungal infection was strongly related to yield (Stynes, 1975). However, the actual incidence of fungi recorded by Stynes (1975) appeared to be so low that some indirect relationship between yield and fungal infection was undoubtedly involved. Price (1970), in Victoria, detected *Pythium*

spp., *R. solani*, *Fusarium* spp., *Bipolaris sorokiniana* and *G. graminis* within patches of poor growth. *P. neglectus* also seemed to occur more frequently within these patches. In the Upper South East and Mid-North of South Australia, Kimpinski (1972) found that patches in wheat crops contained more *G. graminis*, *R. solani* and *P. neglectus* than the surrounding healthy crop. His inoculation experiments proved the earlier hypothesis of de Beer (1965) that *R. solani* and *P. neglectus* can act synergistically to cause the observed damage in South Australian cereal crops.

In the work reported here, the health and number of lateral roots along main root axes was improved by treatment with methyl bromide and aldicarb, although the fumigant tended to be more effective than the nematicide in this role. This may be because methyl bromide kills fungi as well as *P. neglectus* in the soil. Growth of lateral roots was thus enhanced more by the fumigant than by the nematicide, although isolations of fungi were not made directly from the laterals themselves. Under both chemical treatments, primary laterals had in turn branched to produce secondary laterals, which were observed only rarely under natural field conditions in the Murray Mallee.

Weaver (1926) determined that spring wheat could produce between five and nine lateral roots per inch (ie. 2.0 - 3.6/cm), and winter wheat twelve or more per inch (ie. 4.8/cm) of main root axis. These laterals were scattered irregularly along the root system, but were most abundant in the top 60 cm of the soil. Lateral frequency measured in 1989 was up to 0.49/mm of root for untreated areas and as many as 0.76/mm for fumigated areas. The former is identical to Weaver's (1926) measurement for winter wheat (ie. 0.48/mm), and the latter is nearly double that of Weaver's (1926) value.

Fungi alone have been reported to damage lateral roots of various plants, as discussed in Chapter 6, but nematodes, either by themselves or in combination with fungi, also damage these roots. *Rhizoctonia fragariae* and *P. penetrans* significantly reduce the length of strawberry lateral roots (LaMondia and Martin, 1988, 1989). Corbett (1972) observed *P. fallax* in wheat, feeding in cells at the base of laterals, at the junction of main and lateral roots, and on lateral root initials, as well as at the tips of these roots. This caused browning at lateral bases, discolouration of the tips and brown lesions, producing stunted and necrotic laterals by ten weeks. Unlike *P. thornei*, which does not

prefer any specific part of the wheat root system (Baxter and Blake, 1967), *P. fallax* prefers to feed on main root axes at points where laterals emerge (Corbett, 1972). According to Corbett (1972), this is due to the formation of openings in the cortex made when laterals emerge, attracting nematodes to substances diffusing from rapidly growing or damaged cells. Zunke (1990) found that *P. penetrans* were also attracted to points where laterals emerge from the main root axes, and juveniles, especially, fed on or in the lateral roots.

Reduction in the number of lateral roots may indicate the effect of *P. neglectus* on root growth, but measurements of lateral length may be even more enlightening. In most cases observed in the work reported here, laterals are produced, but subsequently become discoloured and stunted. Plants from untreated areas, in the field, display this damage. Experiments with chickpeas, which will be discussed in Chapter 12, indicated that *P. neglectus* had little effect on the number of lateral roots produced, but their length was drastically reduced. Nevertheless, when cortical rotting reaches an advanced stage, root axes are rendered devoid of laterals along the decorticated segments. Weaver (1926) measured laterals on winter wheat to an average length of one inch (2.5 cm), and on some parts of the root system abundant secondary lateral branches had formed. In 1989, only wheat grown on treated areas produced laterals approaching this length, and these were the only plants that had formed any secondary laterals.

Aldicarb effectively reduced lesioning in September, but had little effect in October, whereas methyl bromide consistently reduced the extent of root damage through September and October. The effect of other soil-borne organisms, as well as climatic and edaphic conditions, would have obscured the beneficial effects of nematicide treatment by this stage. Aldicarb is less effective in sandy soils with low organic content (Barker *et al.*, 1988), like those soils encountered in the Murray Mallee. Conversely, fumigants are better dispersed in soils with low organic and clay contents (Raski *et al.*, 1983). Nematodes may also have moved up the soil profile into the root zone of the area previously treated with aldicarb, especially as plants were sown in August after *P. neglectus* numbers had built up on the original crop sown several months previously. Root lesion nematodes occur most frequently at depth in the soil, below cultivation depth

(Thompson *et al.*, 1981), with populations of *P. thornei* in Queensland being highest between 15 cm and 60 cm (Thompson and Clewett, 1989). *P. thornei* has been found to occur at depths of up to 120 cm on the deep soils of New South Wales (Doyle *et al.*, 1987). Aldicarb affects populations of *P. thornei* to a depth of only 15 cm, whereas methyl bromide can control these nematodes to depths of 30 - 90 cm (Doyle *et al.*, 1987). *P. neglectus* populations at depth may then have moved up into the root zone to feed on the relatively healthy root tissues of plants growing in soil previously treated with aldicarb.

Most plant growth variables measured were enhanced more effectively by methyl bromide than by aldicarb, for the reasons already discussed. However, aldicarb actually reduced root length in some instances, and may have had a phytotoxic effect. This was observed at a third site in the Murray Mallee, sown at the same time as the Mannum and Cambrai sites, but was not sampled in 1989, because plants had died as a result of aldicarb application. Conversely, aldicarb can stimulate plant growth regardless of its effect on nematodes (Barker *et al.*, 1988; Barker and Powell, 1988). This may have been the reason that leaf length tended to be enhanced by the nematicide to a greater extent than by the fumigant, although this was not reflected in the shoot dry weight or tiller number. Leaf length, especially at early growth stages, may have been a misleading characteristic to measure.

The effects of methyl bromide were measurable in September and mid-October, but the efficacy of this chemical seemed to have diminished by the end of October. This was particularly obvious in measurements of the shoot dry weight. Factors other than infection of roots by soil-borne organisms would have limited plant growth as the season progressed. Availability of water and nutrients undoubtedly restricted plant growth by late October, especially as plants were not sown until late August, and the plant density after establishment was much greater on the fumigated areas. Plants with healthier root systems and more luxuriant shoot growth have a higher transpiration rate and thus greater water demand, leading to water stress later in the season. The advantage of chemical treatments diminished as the season progressed, with seedlings early in the season exhibiting the greatest benefit from these treatments. It was noted, when sampling, that

soil from the fumigated areas appeared drier than that from adjacent untreated areas, further demonstrating the higher water demand of healthier plants. The surrounding crop, planted several months before, may also have affected the supply of water available for seedlings on the plot area.

Bacteria undoubtedly contribute to the rotting of wheat roots that are already damaged by nematodes and fungi, although the role of bacteria in cereal root disease has never been thoroughly investigated. Numerous bacteriophagous nematodes were present in nematode extractions from the roots, where they were probably feeding on bacteria associated with the rotted root tissues. Moen and Harris (1985) showed that bacteria isolated from cereal roots exacerbated root damage caused by *B. sorokiniana* or *R. solani.* However, these bacteria were also isolated from healthy roots, and none of the bacteria tested reduced root length of wheat plants when acting alone.

The possibilities for interactions between soil-borne organisms within roots are enormous, and these organisms must be studied in controlled inoculation experiments to determine their respective roles in the development of root disease. Species of *Fusarium* and *Pythium* (of which there are many that infect cereals), *M. bolleyi* and *R. solani* are the obvious candidates to test in this manner, in conjunction with *P. neglectus*. Although in previous inoculation experiments (Chapter 7), *M. bolleyi* caused negligible damage to wheat plants, combined with *P. neglectus* the fungus may be more damaging than originally believed. As discussed, nematodes can render plants susceptible to damage by fungi that are not usually pathogenic, and this phenomenon should be investigated in relation to *M. bolleyi*. Furthermore, *M. bolleyi* and *Fusarium* spp. were found to infect roots with damaged laterals significantly more than those with healthy laterals (Chapter 6). One of the effects of *P. neglectus* on plants is its deleterious impact on health and frequency of lateral roots, so *M. bolleyi* and the fusaria may be involved in causing this damage.

There were too few replicates to show, consistently, that *P. neglectus* has a significant effect on plant growth. Even with increased replication, a system involving plants, soil-borne organisms, climatic and edaphic factors (and, consequently, interactions between all these) inherently involves enormous variation. Even in pot

experiments, under controlled conditions, using a range of concentrations of surface-sterilised nematodes with many replicates, the inherent variability of plants and nematodes makes it difficult to show that nematodes have any significant effect on plant growth (Pitcher *et al.*, 1960). Nematodes restrict root growth, but reductions in shoot growth are only obvious under conditions of limited water and nutrient supply. In pots, containing a small volume of soil, it is quite difficult to reproduce field conditions. If water or nutrient availability is restricted in pots, the plants die and, consequently, the effect of nematodes on plant growth cannot be determined. Stanton (1983) found it difficult to demonstrate varietal tolerance to *H. avenae* when plants were grown in tubes of soil, partly due to these factors. Experimental conditions therefore need to be manipulated and strictly controlled to determine accurately the role of *P. neglectus* in root rots of wheat.

CHAPTER 11

THE EFFECT OF *PRATYLENCHUS NEGLECTUS* AND A *PYTHIUM* SP. ON THE GROWTH OF WHEAT

11.1 INTRODUCTION

Field experiments conducted in 1989 suggested that *Pratylenchus neglectus* plays an important role in the rotting of wheat roots (Chapter 10). Concomitant infection of roots by one or more species of fungi may exacerbate the severity of damage caused by these nematodes. Inoculation experiments were therefore undertaken to determine the effect of *P. neglectus* and a species of *Pythium*, by themselves and in combination, on the growth and health of Machete wheat.

Fungi other than *Pythium* spp. may be involved in this complex disease, but the pythia were among the fungi most frequently isolated from field samples in areas of the Murray Mallee infested with *P. neglectus* (Chapter 10). Furthermore, oospores of *Pythium* spp. were often observed in stained roots containing the nematode. The combined effects of *Pythium* spp. and *P. neglectus* on wheat have not been investigated previously, and few cases are documented where pythia and nematodes are known to interact as the causal agents of root disease.

Bratoloveanu (1985) and Singh (1984), in South Australia, studied the possibility that *P. thornei* and *Pythium irregulare* act synergistically to cause cereal root disease, but their inoculation experiments provided no conclusive evidence for a significant interaction between the pathogens in wheat or barley roots. Actually, *P. thornei* is rare on cereals in South Australia, except on the heavier soils in some districts, but does occur in cereal plots at the Waite Institute, and has been reported from localised areas of the Mid-North and Eyre Peninsula of this State.

11.2 METHODS

11.2.1 Experiment 1: *Inoculation with Pratylenchus neglectus*

Surface-sterilised, pre-germinated Machete wheat seeds were sown, one per pot, in Hygienic Lily® plastic cups with a capacity of approximately 650 g of soil. The soil was obtained from the Murray Mallee and steam-sterilised prior to being used.

Nematodes for inoculation were collected from the roots of plants grown in pots of infested Murray Mallee field soil. *P. neglectus* were extracted from the roots by misting, as described in the General Methods. Prior to inoculation, these nematodes were washed several times with SDW in a sintered glass funnel (grade 4) under suction, but the nematodes were not aseptic. Suspensions were prepared in SDW, and nematodes added directly onto the seed at the rates of 100, 1000 or 5500 per plant. Each pot received 1.0 ml of the appropriate concentration of *P. neglectus*, and 1.0 ml of SDW was added to each control pot. Five replicate pots were planted for each treatment. Seed was covered with soil to give a seeding depth of 2.0 cm, and the soil tamped down. The soil had been moistened before planting, so no additional water was applied to pots until four days later. This reduced the risk of nematodes being washed down through the soil before they had a chance to invade the roots. Pots were placed in the glasshouse, in a waterbath maintained at 15±2°C, and each pot watered with distilled water as required.

Plant height was measured each week for seven weeks. After eight weeks, soil was washed from the roots under running tapwater, and plants spread on a white tray, in distilled water, to investigate symptoms on the roots. Roots were misted to extract nematodes, as described in the General Methods, and the number of *P. neglectus* per plant counted. Roots and shoots were dried and weighed.

11.2.2 Experiment 2: *Inoculation with Pythium sp.*

Pre-germinated Machete wheat seeds were sown, one per pot, in the same way as described in Experiment 1.

A *Pythium* sp. previously isolated from field samples was grown in liquid culture, and inoculum of this prepared as described in the General Methods. The species of

Pythium was not determined, but colonies of the isolate grown on plates of PDA had the "rosette" colony formation. Hyphal homogenate, in SDW, was added to pots at the rates of 2.0, 5.0 and 10.0 ml per plant. Each plant received a total of 10.0 ml of liquid, and 10.0 ml of SDW was added to control pots. Five replicate pots were planted for each treatment. Soil was placed above the seed to give a seeding depth of 2.0 cm. Growing conditions were identical to those described for Experiment 1.

Plant height was measured each week for seven weeks. At the end of week eight, soil was washed from the roots under running tapwater. Symptoms on the roots were described, and twelve 1.0 cm root segments removed (at random) from each plant for plating on *Pythium* isolation medium. Roots and shoots were dried and weighed.

11.2.3 Experiment 3: *Inoculation with Pythium sp., Pratylenchus neglectus or both*

After determining the effect of *P. neglectus* and the *Pythium* sp. on wheat plants, a more detailed experiment, using higher inoculum levels, was conducted.

Surface-sterilised, pre-germinated Machete wheat seeds were sown in the same soil as that used in Experiments 1 and 2. Moist soil was placed in lengths of electrical conduit 125.0 mm long and 32.0 mm in diameter, and one seed sown in each tube.

Pythium and *P. neglectus* inocula were prepared as in Experiments 1 and 2. Plants were inoculated with *Pythium* at the rates of 0, 2.0, 10.0 or 20.0 ml of hyphal homogenate per plant, and *P. neglectus* at 0, 500, 1500 or 6000 nematodes per plant. These were inoculated onto plants in all possible combinations, to give sixteen treatments, and each treatment was replicated five times. Tubes received 20.0 ml of the appropriate concentration of *Pythium* homogenate, plus 1.0 ml of nematode suspension. Soil was placed above the seed, resulting in a sowing depth of 2.0 cm.

Tubes were held upright in trays of coarse sand, with the sand covering only the lower 5.0 cm of each tube. These were placed in the glasshouse, and watered with distilled water as required. For the first two weeks, tubes were watered from the top with only small quantities of water, but as water demand increased, water was added to the sand below. This also reduced the risk of nematodes and the fungus being washed into

the sand, as roots were free to grow through the open ends of the tubes.

After eight weeks, soil was washed from roots under running tapwater. Plants were spread on a white tray, in distilled water, and symptoms on the roots described. Frequency of lateral roots was determined by selecting one seminal root axis at random and counting the number of laterals per centimetre of root. Six 1.0 cm root segments were removed, at random, from each plant for plating on *Pythium* isolation medium. Roots were then misted to extract *P. neglectus* from the roots, and the number of nematodes extracted from each plant determined. Roots and shoots were dried and weighed.

11.3 RESULTS

11.3.1 Experiment 1: *Inoculation with Pratylenchus neglectus*

Symptoms

Roots of inoculated plants had sustained little damage (Table 11.1), but only one replicate at each inoculum level was completely free of symptoms. Localised areas of the crown root system exhibited lesioning and decortication (Plate 11.1) similar to that observed on field-grown plants (Chapter 9; Plate 9.4A).

Seminal roots of plants inoculated with 100 *P. neglectus* displayed more severe damage than those at higher levels of inoculum, and these were the only plants with decorticated seminal roots (Table 11.1). Very few seminal roots had distinct cortical lesions, and only 100 *P. neglectus* caused this symptom.

Only the plants inoculated with 100 *P. neglectus* had decorticated crown roots (Table 11.1; Plate 11.1). The number of crown roots that were stunted with orange-brown cortical discolouration increased up to the 1000 inoculum level. Several crown roots on plants inoculated with 100 nematodes were reduced to orange-brown, rotted "spears". Only the plants inoculated with 5500 *P. neglectus* had crown roots with distinct cortical lesions.

With all the levels of inoculum, the crown roots were damaged more severely than the seminal or lateral roots (Table 11.1). Discolouration and stunting of lateral roots was

only observed with the highest level of nematode inoculum.

TABLE 11.1: Incidence of symptoms on seminal, crown and lateral (LAT) roots of Machete wheat plants inoculated with *Pratylenchus neglectus* (nematodes/plant). Symptoms were assessed on five replicates, eight weeks after inoculation, for each treatment. (Disc. = discoloured; Decort. = decorticated; - denotes absence of symptom; + denotes frequency of symptom).

	LAT	SEMINAL ROOTS			CROWN ROOTS			
Inoculum (nematodes /plant)	Disc., stunted	Disc.	Flaccid, decort.	Lesioned	Orange-brown, stunted	Flaccid, decort.	Orange-brown, "spears"	Lesioned
0	-	+	-	-	+	-	-	-
100	-	-	++	+	++++	++++	-	-
1000	-	-	-	-	++++++	-	+++	-
5500	++	-	-	-	++++	-	-	+

Plant Growth

Inoculation with *P. neglectus* had little significant effect on the plant height over seven weeks, although inoculation with 1000 and 5500 nematodes consistently resulted in plants being shorter than the uninoculated controls (Table 11.2). Significant differences between treatments occurred only at one week, reflecting the reduced vigor of inoculated plants at emergence. At one week, 100 nematodes reduced plant height by 21% and 5500 by 19%. At other times, plant height was reduced by only 2 - 10%.

No level of inoculum significantly reduced root or shoot dry weight. However, inoculation with 5500 *P. neglectus* led to a 6% decrease in shoot weight, and a 15% reduction in root mass (Table 11.3). The absence of statistically significant reductions in plant growth may be a result of the inadequate number of replicates on which these variables were measured. However, the nematodes seem to have had a greater effect on root growth than they did on shoot growth.

PLATE 11.1: Crown roots of Machete wheat that had been inoculated with *Pratylenchus neglectus*. Segments of root were lesioned and decorticated (compare with Plate 9.4A).

Plate 11.1

TABLE 11.2: The effect of *Pratylenchus neglectus* (nematodes/plant) on the height (mm) of Machete wheat plants over seven weeks. (Values are the mean of five replicates for each treatment).

	INOCULUM (nematodes/plant)				
Weeks	**0**	**100**	**1000**	**5500**	**LSD (0.05)**
1	93.4	92.8	73.6	75.6	16.6
2	167.2	170.6	150.6	153.4	25.2
3	214.0	228.6	199.4	198.8	24.1
4	238.2	256.6	223.0	231.6	29.4
5	287.6	304.4	282.0	291.8	25.7
6	316.8	318.8	306.4	319.4	22.6
7	341.0	328.6	331.4	334.6	24.1

TABLE 11.3: The effect of *Pratylenchus neglectus* (nematodes/plant) on shoot and root growth (mg/plant) of Machete wheat plants, and number of nematodes extracted from eight week old plants. (Values are the mean of five replicates for each treatment).

Inoculum (nematodes/plant)	**Shoot Dry Weight (mg/plant)**	**Root Dry Weight (mg/plant)**	**Nematodes/Plant**
0	1063.8	273.2	0
100	1215.0	247.0	149.4
1000	1058.8	273.4	261.3
5500	997.0	233.0	761.3
LSD (0.05)	465.6	117.3	264.2

Nematode Extraction

At the end of eight weeks' growth, *P. neglectus* were extracted from the roots of all inoculated plants (Table 11.3), where they had been feeding and laying eggs within root cortices. Plants inoculated with 5500 *P. neglectus* contained significantly ($P<0.05$) more nematodes than those inoculated with either 100 or 1000. Control plants were not infected with *P. neglectus*.

As inoculum level increased, the invasion and apparent reproduction rate of the nematodes decreased (Table 11.3). At the 100 inoculum level, nematode density increased 1.5 times, while inoculation with 1000 and 5500 nematodes led to final populations of only 0.3 and 0.1 times the initial inoculum density, respectively. Fewer nematodes were extracted from plants at the higher inoculum levels than were originally added to the pots.

11.3.2 Experiment 2: *Inoculation with Pythium sp.*

Symptoms

The *Pythium* sp. damaged roots less than *P. neglectus* did in Experiment 1. Only plants inoculated with 5.0 or 10.0 ml of hyphal homogenate sustained root damage, with plants inoculated with 5.0 ml showing more visible symptoms on the roots than those inoculated with 10.0 ml (Table 11.4). Unlike *P. neglectus*, the *Pythium* sp. caused small (<1.0 mm), but distinct, brown cortical lesions. Some root tips were brown, soft and rotted for a length of 1.0 - 3.0 mm. Lateral roots were, apparently, not affected by *Pythium* inoculum, nor did roots suffer any decortication (Table 11.4).

Plant Growth

The *Pythium* sp. affected plant height less than did inoculation with *P. neglectus*, and the fungus did not significantly reduce plant height at any time over the seven weeks. At two and three weeks, 2.0 ml or 10.0 ml of inoculum reduced plant height by only 3 - 7% (Table 11.5). After five weeks, all levels of inoculum reduced shoot height, but by only 3 - 4%. The height of plants was not reduced by the *Pythium* sp. at one, four, six or seven weeks.

Only the highest rate of *Pythium* inoculum was associated with decreased shoot or root growth of eight week old plants, although neither was statistically significant (Table 11.6). Root mass was decreased by 9%, and that of shoots by 5%. As with plant height, root and shoot dry weight were affected less by *Pythium* than they were by *P. neglectus*.

TABLE 11.4: Incidence of symptoms on roots of Machete wheat plants inoculated with *Pythium* sp. (ml hyphal homogenate/plant). Symptoms were assessed on five replicates, eight weeks after inoculation, for each treatment. (- denotes absence of symptom; + denotes frequency of symptom).

Inoculum (ml hyphae/plant)	Dark brown lesions	Dark brown root tips	Light brown lesions	Light brown root tips
0	-	-	-	-
2.0	-	-	-	-
5.0	+++	++++	-	++
10.0	-	-	++	-

TABLE 11.5: The effect of a *Pythium* sp. (ml hyphal homogenate/plant) on the height (mm) of Machete wheat plants over seven weeks. (Values are the mean of five replicates for each treatment).

	INOCULUM (ml of *Pythium* hyphae/plant)				
Weeks	0	2.0	5.0	10.0	LSD (0.05)
1	112.2	114.2	117.6	112.4	21.1
2	190.0	183.2	200.8	176.2	30.8
3	313.8	301.4	316.6	304.2	39.9
4	341.8	342.0	349.4	341.4	40.4
5	401.6	387.0	390.0	385.8	58.5
6	412.2	444.8	423.8	429.8	40.3
7	417.4	452.8	446.8	444.2	40.5

Pythium Infection

All inoculated plants were infected by the *Pythium* sp. when sampled at eight weeks. The percentage of root samples infected increased with increasing inoculum concentration (Table 11.6). Root samples from plants inoculated with 2.0, 5.0 and 10.0 ml of hyphae had 28%, 43% and 58% infection, respectively. Inoculation with 10.0 ml of hyphae resulted in significantly ($P<0.05$) more root samples being infected than did inoculation with 2.0 ml of hyphae.

A very small percentage of roots from the uninoculated pots were also infected (Table 11.6), due to the introduction of *Pythium* from some external source. The water, although distilled, may have contained *Pythium* oospores. It is more probable that air-borne inoculum infected soil before or after planting, especially as Adelaide experienced two "dust storms" at the time these experiments were conducted.

TABLE 11.6: The effect of *Pythium* sp. (ml hyphal homogenate/plant) on shoot and root growth (mg/plant) of Machete wheat plants, and frequency of *Pythium* (% root segments infected) isolated from eight week old plants. (Values are the mean of five replicates for each treatment).

Inoculum (ml hyphae/plant)	Shoot Dry Weight (mg/plant)	Root Dry Weight (mg/plant)	% Roots Infected
0	1978.4	421.6	3.3
2.0	2097.4	540.8	28.3
5.0	2086.0	435.2	43.3
10.0	1875.8	382.2	58.3
LSD (0.05)	438.4	197.3	24.1

11.3.3 Experiment 3: *Inoculation with Pythium sp., Pratylenchus neglectus or both*

Symptoms

Seminal and crown roots of plants, inoculated with either organism or both, suffered varying levels of discolouration, cortical lesioning and decortication, with both root systems displaying "spiked" root tips (Table 11.7). Lateral roots were shortened, and their frequency along seminal root axes reduced. Seminal roots were discoloured more severely than the crown roots, with both *P. neglectus* and *Pythium* contributing to this damage.

There was a tendency for the incidence of discolouration to increase with

increasing *P. neglectus* inoculum, with this trend more pronounced on the seminal than crown roots (Table 11.7). Lesioning of seminal roots was more prominent when plants were inoculated with both organisms, while crown roots had very few lesions with any inoculation treatment.

Decortication of roots occurred with *P. neglectus* by itself, but rarely when plants were inoculated with *Pythium* alone (Table 11.7). Seminal roots were decorticated more than crown roots. Both root systems had "spiked" root tips, especially when inoculated with 500 or 6000 *P. neglectus*, regardless of the concentration of *Pythium* inoculum. There was a tendency for lateral roots to appear shortened with all treatments, except at the highest level of *Pythium* inoculum.

Plant Growth

Inoculation with *Pythium* by itself only reduced root dry weight significantly ($P<0.05$) at the highest inoculum level, with root weight being 55% below that of the control (Figure 11.1). All levels of *P. neglectus* reduced root weight, although none significantly, and there was a tendency for the 500 nematode inoculum to have a greater deleterious effect than the higher rates. *P. neglectus* reduced root weight, on average, by 29%.

P. neglectus plus *Pythium* reduced root growth more than nematodes alone, and in some cases more than the fungus by itself (Figure 11.1). Five hundred *P. neglectus* in conjunction with all levels of *Pythium* inoculum significantly ($P<0.05$) reduced root weight, with reductions between 64% and 77%. The same level of *P. neglectus* by itself reduced root weight by 42%, while *Pythium* alone decreased root growth by 21 - 55%. Inoculation with 1500 nematodes plus the lowest level of *Pythium* reduced root weight by 54%, which was significant ($P<0.05$). These treatments individually decreased root weight by only 13% and 21%, respectively. The highest rate of *P. neglectus*, combined with 10.0 ml of *Pythium*, decreased root weight by 62%, and this was significant ($P<0.05$). These inoculum levels each decreased root weight by 33% and 0%, respectively. Fungus plus nematode significantly reduced root dry weight in some instances, whereas *P. neglectus* by itself did not.

TABLE 11.7: Incidence of symptoms on seminal, crown and lateral roots of eight week old Machete wheat plants inoculated with *Pratylenchus neglectus* and *Pythium* sp. (Inoculum = ml of *Pythium* hyphal homogenate plus number of nematodes/plant; - denotes absence of symptom; + denotes frequency of symptom; tr = trace; Disc = discoloured; Les = lesioned; Decort = decorticated; ST = "spear" root tips; SL = shortened laterals). "Incidence" indicates how often each symptom appeared over the whole root system, as a total for the five replicates (ie. assessment on five plants); there were no plants on which all roots were damaged.

Inoculum fungus(ml) + nemas(#)	SEMINAL ROOTS				CROWN ROOTS				LATERALS
	Disc	Les	Decort	ST	Disc	Les	Decort	ST	SL
0 + 0	tr	tr	-	-	tr	tr	tr	-	tr
0 + 500	+++	-	-	+	tr	-	+	tr	+
0 + 1500	++++	tr	+	-	-	tr	-	-	tr
0 + 6000	+	tr	tr	++	+	-	-	+	++
2 + 0	+	+	-	-	++	-	-	-	tr
2 + 500	+	tr	tr	+	+	-	-	tr	tr
2 + 1500	+	+	-	-	tr	tr	tr	-	tr
2 + 6000	+++	+	tr	tr	+	-	tr	tr	+
10 + 0	++	tr	tr	-	tr	-	-	-	+
10 + 500	++	+	tr	tr	tr	tr	-	++++	tr
10 + 1500	++	tr	-	-	tr	tr	-	-	+++
10 + 6000	++++	++	-	++	+	tr	-	tr	+
20 + 0	++	-	-	+	tr	-	-	-	tr
20 + 500	+	-	-	+	-	-	-	+	-
20 + 1500	+	tr	-	-	tr	tr	-	++	-
20 + 6000	++	+	+	-	+	+	-	-	-

Neither *P. neglectus* nor *Pythium*, when alone or in combination, significantly reduced the dry weight of shoots (Figure 11.2). In fact, increasing rates of *Pythium* inoculum tended to increase shoot weight. *P. neglectus* only slightly decreased shoot weight at the highest inoculum level. Both organisms had a greater deleterious effect on root growth than on shoot growth.

All inoculation treatments decreased the frequency of lateral roots along seminal root axes (Figure 11.3). *Pythium* reduced lateral number by 31 - 35%, but this was not

significant for any level of inoculum, nor was this significant for *P. neglectus,* which decreased the frequency of laterals by 21 - 31%. Intermediate levels of nematode inoculum in conjunction with high *Pythium* inoculum reduced lateral root number more than either organism alone. Five hundred or 1500 *P. neglectus* plus 10.0 ml of *Pythium* significantly ($P<0.05$) reduced lateral root frequency. These reductions were, respectively, 45% and 42%. Five hundred or 1500 nematodes alone reduced number of laterals by 22% and 31%, respectively, with 10.0 ml of *Pythium* decreasing lateral frequency by 35%. Twenty millilitres of *Pythium* inoculum, combined with 1500 nematodes, reduced lateral root number by 47%, which was significant ($P<0.05$). The organisms in this case individually reduced lateral root number by 32% and 31%, respectively.

Nematode Extraction and Pythium Infection

The invasion and reproduction rates of *P. neglectus* in small diameter tubes were very low (Figure 11.4), despite high inoculum levels, although these did not decrease with increasing inoculum levels as was observed in pots. Inoculation with 500 and 1500 nematodes resulted in 0.3 times this number after eight weeks, and inoculation with 6000 gave 0.4 times this number at the end of the experiment. This may, in part, be due to incomplete extraction of nematodes during the misting process. Some roots were stained after being misted, and *P. neglectus* could still be detected in the root cortices.

Although *Pythium* and *P. neglectus* apparently caused more damage to roots than did either organism by itself, all levels of *Pythium* inoculum significantly ($P<0.05$) decreased the number of nematodes recovered from the roots of plants inoculated with 6000 *P. neglectus* (Figure 11.4). Between 10% and 43% of these root segments were infected with the fungus. Similar fungal infection frequencies were recorded on the roots of plants at lower nematode inoculum levels (Figure 11.4). Abundant *Pythium* oospores were observed in root segments that were stained with lactoglycerol-cotton blue.

FIGURE 11.1: Root dry weight (mg/plant) of Machete wheat plants eight weeks after inoculation with *Pratylenchus neglectus* at the rates of 0, 500, 1500 or 6000 nematodes/plant, in conjunction with 0, 2.0, 10.0 or 20.0 ml of *Pythium* sp. hyphal homogenate. (Values are the mean of five replicates for each treatment). LSD (0.05) = 54.6.

FIGURE 11.2: Shoot dry weight (mg/plant) of Machete wheat plants eight weeks after inoculation with *Pratylenchus neglectus* at the rates of 0, 500, 1500 or 6000 nematodes/plant, in conjunction with 0, 2.0, 10.0 or 20.0 ml of *Pythium* sp. hyphal homogenate. (Values are the mean of five replicates for each treatment). LSD (0.05) = 104.0.

FIGURE 11.3: Number of lateral roots/cm of seminal root axes on eight week old Machete wheat plants inoculated with *Pratylenchus neglectus* at the rates of 0, 500, 1500 or 6000 nematodes/plant, in conjunction with 0, 2.0, 10.0 or 20.0 ml of *Pythium* sp. hyphal homogenate. (Values are the mean of five replicates for each treatment). LSD (0.05) = 1.4.

FIGURE 11.4: Number of *Pratylenchus neglectus* extracted from roots of Machete wheat eight weeks after inoculation with *P. neglectus* at the rates of 0, 500, 1500 or 6000 nematodes/plant, in conjunction with 0, 2.0, 10.0 or 20.0 ml of *Pythium* sp. hyphal homogenate. Levels of *Pythium* sp. infection (% root segments infected) are also indicated. (Values are the mean of five replicates for each treatment). LSD (0.05) = 574.3.

Figure 11.1

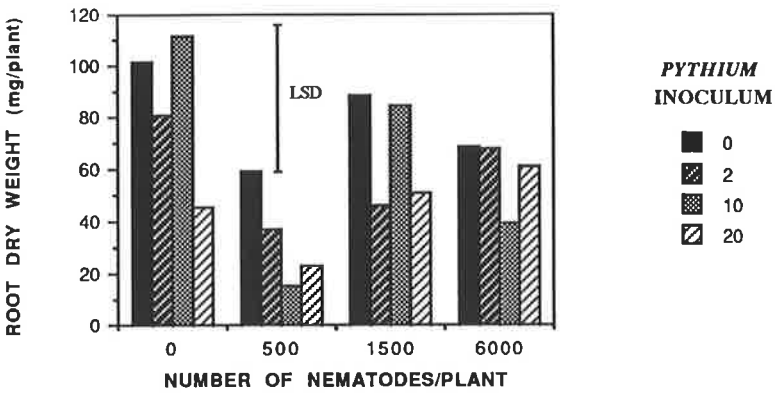


Figure 11.2

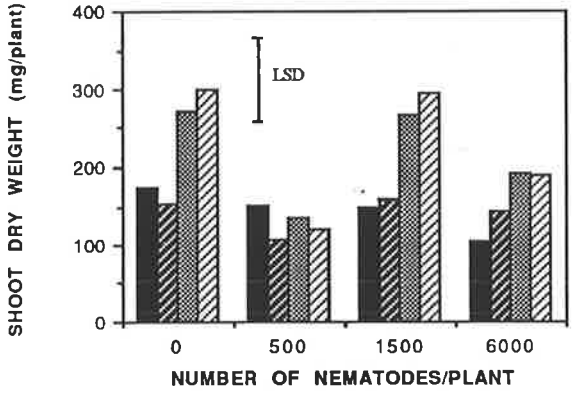


Figure 11.3

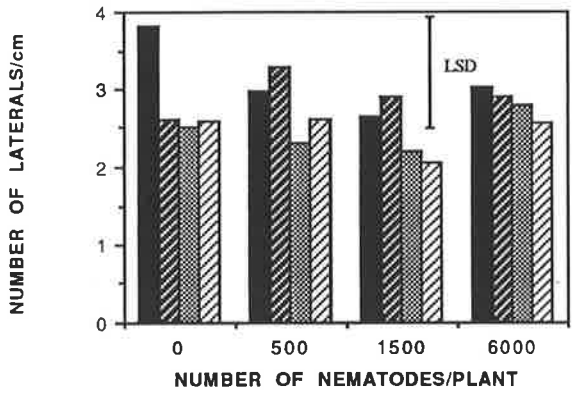
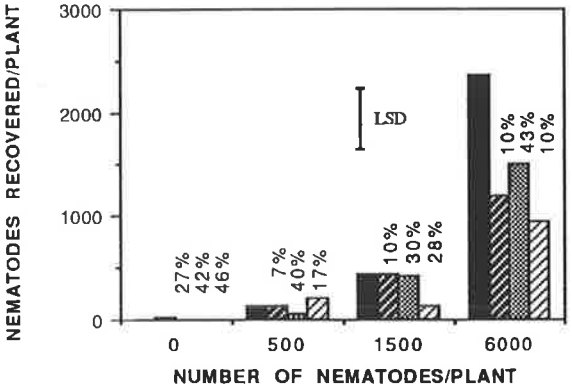


Figure 11.4



11.3.4 Summary of Results

Inoculation of Machete wheat with a *Pythium* sp., *Pratylenchus neglectus*, both or neither showed that:

(1) At the inoculum levels used in these experiments, *P. neglectus* by itself caused only slight damage to roots.

(2) Inoculation with *P. neglectus* had little effect on plant height, and no significant effect on shoot or root weight, although the highest level of inoculum reduced shoot weight by 6% and root weight by 15%.

(3) The *Pythium* sp. caused less damage to roots than did the nematode, and infection levels in these experiments were lower than those recorded on Machete wheat in the field (Chapter 10). Unlike *P. neglectus*, the fungus caused distinct cortical lesions and damage to root tips. Lateral roots were apparently unaffected. *Pythium* did not cause decortication of roots.

(4) Inoculation with *Pythium* led to no significant reduction in plant height, root or shoot dry weight.

(5) Plants inoculated with both nematode and fungus suffered more visible damage than those inoculated with either organism by itself.

(6) *P. neglectus* plus *Pythium* reduced root weight more than inoculation with either pathogen by itself, significantly reducing root weight (by up to 77%), while *P. neglectus* alone decreased root weight up to 42% and *Pythium* alone by up to 55%.

(7) Frequency of laterals was reduced by all treatments, but more so by *P. neglectus* plus *Pythium*. The fungus reduced lateral root frequency by 31 - 35% and *P. neglectus* reduced the frequency of these roots by 21 - 31%. Inoculation with both organisms decreased the frequency of laterals by up to 47%.

(8) At the highest level of nematode inoculum, *Pythium* infection significantly reduced the number of *P. neglectus* subsequently extracted from the roots.

11.4 DISCUSSION

It is difficult to show, with inoculation experiments, that nematodes have any significant effect on plant growth, due to the inherent variability of plants and nematodes (Pitcher *et al.*, 1960), and because it is difficult to induce water and nutrient stress in plants grown in pots. Florini and Loria (1990) found that cereals and other hosts inoculated with *Pratylenchus penetrans* were similar in height, shoot dry weight and root fresh weight to the uninoculated controls in all pot experiments. Olthof (1990), however, was able to demonstrate that *P. neglectus* suppressed shoot growth of potato in inoculation experiments. Townshend (1984) found that *P. neglectus* at the rate of 425 nematodes per plant reduced root weight of lucerne (alfalfa; *Medicago sativa*) plants. Griffin and Gray (1990) also reported substantial growth reductions in lucerne due to *P. neglectus*. Plants inoculated with 1000, 5000 or 10 000 nematodes suffered reductions in shoot growth of up to 18%, 32% and 40%, respectively. Five thousand and 10 000 *P. neglectus* decreased root growth of lucerne by 40% and 59%, respectively. In experiments reported here, *P. neglectus* significantly reduced early plant vigor (Table 11.2), but did not significantly reduce shoot or root dry weights (Table 11.3). Due to substantial variation between replicates, few differences between treatments were significant.

Growing conditions and experimental techniques need to be manipulated to reduce the large amount of variation between replicates, and increasing the level of replication would reduce the differences necessary for demonstrating significance. Factors such as the soil water status, particularly in the tubes, need to be better controlled, as moisture conditions affect the motility of nematodes in the soil. Furthermore, plants often need to be subjected to some level of water and nutrient stress, as occurs under field conditions, for the deleterious effects of nematode attack to become obvious. Nematodes used as inoculum were not aseptic, so other organisms may have been introduced to pots, and these may have contributed to the root damage. Surface-sterilised nematodes would have been more suitable for use as inoculum.

Some of the symptoms observed in the field, on the roots of plants growing in

areas infested with *P. neglectus*, were reproduced by inoculation of plants with this nematode. Although only in localised areas, roots were decorticated or "spiked", crown roots were stunted with orange-brown discolouration, and laterals were discoloured and stunted. Infection with *Pythium* caused less damage to roots than did the nematode, but some roots sustained distinct cortical lesions and damage to root tips. However, inoculation with *P. neglectus*, alone or in combination with *Pythium*, did not cause root damage equal to the severity of that observed in the field (Chapter 10), where *P. neglectus* and *Pythium* (among other fungi) were detected in root tissues. The number of *P. neglectus* infecting roots at the higher inoculum levels was comparable to that extracted from field-grown plants, but the frequency of *Pythium* infecting inoculated plants (average 26%) was lower than that isolated from Machete wheat sampled from the field in 1989 (average 75%; Chapter 10). *Pythium* may therefore be causing more damage in the field than that observed in these inoculation experiments. However, several species of *Pythium* occur in the field, but not all are pathogenic. The isolate used in these experiments may have been only weakly pathogenic to Machete wheat. All *Pythium* spp. present in the field need to be identified, and their pathogenicity to wheat varieties determined.

P. neglectus seemed to be the primary incitant of decortication and the production of "spiked" roots. The latter symptom, combined with orange-brown discolouration of cortical tissues, may be confused with symptoms currently attributed, by some workers, to infection with *Rhizoctonia solani*. Infection by this fungus should not be inferred solely from the appearance of symptoms in the field, as *P. neglectus* is invariably involved with this root damage, and can be isolated from the roots (Chapter 10). The problem of diagnosis, however, is then compounded by the fact that *P. neglectus* vacate extensively damaged tissues (Dropkin, 1989) in search of a fresh food source. By the time severe root damage is recognised in the field, the nematodes can often only be found in the relatively healthy roots and in the surrounding soil.

It would seem that plants inoculated with *P. neglectus* and *Pythium* suffered more root damage than those inoculated with either organism alone. Inoculation with both pathogens resulted in larger reductions in root growth and frequency of laterals than did

inoculation with either organism by itself, although the inoculum concentrations of both organisms were identical whether the pathogens were used singly or in combination. This phenomenon was discussed in detail at the end of Chapter 10.

Pythium spp. have been studied on cereals in conjunction with *P. thornei*, but not with *P. neglectus*. Bratoloveanu (1985) and Singh (1984) tested the effects of *Pythium irregulare* and *P. thornei* on wheat and barley in pots, but found no evidence for a significant interaction between the two pathogens. Results from Experiments 1 and 2 indicate that *P. neglectus* had a more deleterious effect on Machete wheat than did the *Pythium* sp., but Bratoloveanu (1985) found that *P. irregulare* had a greater effect on barley than did *P. thornei*. Barley is, however, usually subject to more damage from *Pythium* infection than is wheat.

P. neglectus and *Pythium*, singly or in conjunction, adversely affected root growth more than that of shoots. However, Griffin and Gray (1990) found that root and shoot growth of lucerne (alfalfa) were affected similarly by *P. neglectus*, with reductions in root weight directly related to decreased shoot weight. Cereal root diseases, especially those involving several organisms, are difficult to diagnose from above-ground symptoms, and effects on shoot growth can be difficult to demonstrate in pot trials. Unlike conditions in the field, water and nutrient availability are generally not limiting factors in pots, and plants are thus able to better tolerate infection by nematodes and fungi. In fact, Orion *et al.* (1984) found that *P. mediterraneus* (originally described as *P. thornei*) caused little damage to wheat in Israel unless water was a limiting factor. On the other hand, Bhatt (1986) showed that, at high inoculum levels, *P. thornei* did significantly reduce the height of chickpeas.

Conversely, healthier root systems can support higher nematode populations than small, rotted root systems. However, this was not the case in these tests, where reproduction rates of the nematode were very low because of the short duration of the experiments. *Pratylenchus* spp. tend to reproduce more actively in a stressed host (Dropkin, 1989), which may be, in part, the reason for low reproduction rates in the experiments reported here. Under the conditions of these experiments, levels of damage incurred by roots were probably too low for the effects of infection to be seen in the

growth of shoots. Although the vigor of plants after emergence was significantly reduced by *P. neglectus* (Table 11.2), plants seemed to recover and the overall effect the nematodes had on plant growth was slight.

Plants inoculated with the lowest number of *P. neglectus* (100 per plant) in Experiment 1 actually suffered more damage than those inoculated at the higher levels, and were the only plants in this experiment to exhibit decortication of either seminal or crown roots. This may have been because nematodes at the lower inoculum level experienced less competition for infection sites, and thus more readily invaded the roots. The apparent reproduction rate at this level of inoculum was actually greater than that at the higher inoculum levels. When plants in pots are infected with nematodes, competition often results in lower infection levels and relatively less damage to plants as inoculum levels are increased (J. M. Fisher, personal communication). Griffin and Gray (1990) recorded higher reproduction rates of *P. neglectus* on lucerne (alfalfa) at 1000 nematodes per plant than at either 5000 or 10 000 nematodes per plant.

Reproduction rates of *P. neglectus* were low in both inoculation experiments, although Zunke (1990) determined that *P. penetrans* could lay one egg per day. Number of eggs within the roots at the end of eight weeks was not determined, but many were present in the segments of root that were stained in lactoglycerol-cotton blue after the misting process. Unless eggs hatched during misting, these juveniles would have been overlooked.

In pots, *P. neglectus* had a more deleterious effect on root growth than did *Pythium*, although the same was not true for plants grown in tubes. Both *Pythium* and *P. neglectus* caused greater reductions to root weight in tubes than they did in pots. A wider array of symptoms was also seen on the plants in tubes and the damage incurred by roots was greater. This is probably because higher inoculum levels, per unit volume of soil, were applied to the tubes. Nematodes would have penetrated roots in greater numbers in tubes than in pots.

Unlike *P. neglectus*, inoculation with *Pythium* caused lesions in the cortex of wheat roots. It is accepted that infection with *Pratylenchus* spp. does not result in distinct lesions in root cortices of all hosts (Dropkin, 1989). Corbett (1972) found that *P. fallax*

reproduced better on wheat and barley than on sugar beet, but symptoms developed more slowly and appeared less severe on the cereals than on beet. Cells within wheat roots were, however, severely damaged and had collapsed, although no symptoms showed externally on the roots (Corbett, 1972). Lesions caused by *P. neglectus* are readily observed on the roots of various leguminous hosts, as will be shown in the following chapter.

In Experiment 3, the seminal roots of Machete wheat sustained more discolouration and decortication than did the crown roots. There was a tendency for root discolouration to increase with increasing *P. neglectus* inoculum levels, regardless of *Pythium* inoculum, and this was more pronounced on seminal than on crown roots. Seminal roots of wheat generally support higher *P. neglectus* populations than do crown roots (Kimpinski, 1972; Kimpinski *et al.*, 1976; Patel, 1983; Stynes and Veitch, 1983), and this may indicate that seminal roots have some tolerance to *P. neglectus*, hence the high numbers in these roots. As seminal roots are produced before the crown roots, they are invaded early in the season, and this early infection is most damaging to plants (Patel, 1983), partly because of the extended duration of this type of infection. Seminal roots are thus vulnerable to invasion by fungi, although plants can recover to some extent once crown roots are produced (Van Gundy *et al.*, 1974), and can then tolerate large populations of *P. neglectus* in the seminal roots (Kimpinski, 1972). However, in the field, *P. neglectus* invade new crown roots as these are produced, with concomitant infection by fungi causing severe damage to these roots (Chapters 9 and 10). In contrast to the field observations, seminal roots in Experiment 3 were lesioned more than the crown roots. This is probably explained by the low infective and reproductive rates of the nematode in seminal roots in this experiment. Few nematodes would have migrated from seminal to crown roots, subjecting crown roots to little damage.

Infection with *Pythium*, at the highest level of *P. neglectus* inoculum, significantly reduced the number of nematodes subsequently extracted from the roots. Furthermore, lateral roots were shortened by all treatments, except at the highest level of *Pythium* inoculum. Fungal infection can interfere with nematode establishment and reproduction within roots (McKeen and Mountain, 1960; Patel, 1983; Singh, 1984; Walia and Gupta,

1986). This was probably the case in Experiment 3, although concomitant infection with *Pythium* and *P. neglectus* still caused more damage to plants than did inoculation with either organism by itself. The reverse is generally untrue (namely, that nematodes reduce fungal infection) and nematode invasion often increases infection of roots by fungi, as discussed in the previous chapter. However, it has been noted that the reproduction rate of *P. neglectus* on peppermint is greater when plants are also infected with *Verticillium dahliae* (Faulkner and Skotland, 1965; Faulkner *et al.*, 1970).

Experiment 3 did not demonstrate conclusively a synergistic interaction between *Pythium* and *P. neglectus*. The effects of these organisms on wheat may be additive rather than synergistic, although more root damage was observed when both organisms were present. It seems that infection of roots with *P. neglectus* and one or more fungal species is needed for full disease expression.

CHAPTER 12

INFECTION OF CEREAL AND LEGUME VARIETIES WITH *PRATYLENCHUS NEGLECTUS*

12.1 INTRODUCTION

Pratylenchus neglectus has a very wide host range, including legumes as well as cereals and other crop species. Apart from the nematodes' direct effect on the growth of various hosts, different species and varieties will affect nematode populations in the soil, therefore influencing the performance of subsequent crops. This has serious implications when planning crop rotation strategies. This aspect of *P. neglectus* biology has not been investigated previously in South Australia, although the influence of different crops on *P. thornei* populations has been studied in Queensland (Thompson, 1987) and Syria (Greco *et al.*, 1988).

Chemical treatments such as aldicarb (Temik®), are generally effective in controlling nematodes, but are not cost-effective for use in broadacre agriculture (Van Gundy *et al.*, 1974; Greco *et al.*, 1988; Townshend, 1989). Nematicides are also very dangerous chemicals, so control measures that are economical, while having a minimum, negative impact on the environment are desirable (Van Gundy *et al.*, 1974).

Cultivation can reduce nematode numbers in the soil (Thompson and Clewett, 1989), but is not completely effective because root lesion nematodes tend to occur at depth in the soil (Doyle *et al.*, 1987; Thompson and Clewett, 1989), below the depth of cultivation (Thompson *et al.*, 1981). Stynes and Veitch (1983) reported that *P. neglectus* numbers in crops on the Yorke Peninsula of South Australia were higher when little cultivation was undertaken prior to cropping. In Queensland, Thompson *et al.* (1981) also found that *P. thornei* populations were greater after zero tillage than when conventional cultivation practices were carried out.

Crop nutrition also influences plant response to nematode infection, as well-fertilised crops can better withstand attack by pests. Host vigor can be improved by the

application of nitrogenous fertiliser (Van Gundy *et al.*, 1974), but healthy crops that can tolerate infection leave large populations of *Pratylenchus* in the soil (Thompson *et al.*, 1981), which in turn invade the next susceptible crop sown. However, *Pratylenchus* spp. often reproduce more actively in a stressed host (Dropkin, 1989), and roots that have been damaged, whether by fungi or nematodes, cannot take advantage of an improved soil nutrient status. Adequate water availability also improves host vigor, thus increasing nematode activity, but this aspect of crop production is beyond the control of South Australian cereal farmers. A complex relationship exists between host vigor and nematode populations, with many interacting factors involved.

Two separate genetic mechanisms control the interaction between the host species and the pest nematode. Tolerance/sensitivity refers to the ability of the host to withstand invasion by the pest, while resistance/susceptibility is used to describe the effects on nematode populations. Breeding resistant crop varieties is the desirable course to take in controlling *P. neglectus*. Tolerance to the nematode has advantages for the current, tolerant crop, but still leaves high populations of *P. neglectus* in the soil to infect the next susceptible crop sown. Townshend (1989) detected differences between oat cultivars in their resistance to *P. neglectus* or *P. penetrans*, while in Mexico, Van Gundy *et al.* (1974) found no resistance to *P. thornei* in the 30 commercial wheat varieties or breeding lines tested, but they reported some useful levels of tolerance that could be incorporated into breeding programs. In Australia, O'Brien (1983) reported that all wheat varieties tested were susceptible to *P. thornei*, but there were differences between varieties in their reaction to nematode infection. Breeding for tolerance to *P. thornei* is underway in Queensland, where incorporation of tolerance from Mexican wheat varieties has produced some promising genetic material. This program also aims at selecting wheat varieties that maintain low nematode populations in the soil (Queensland Wheat Research, 1988).

Resistance to *P. neglectus* has not been recognised in South Australian wheat varieties, but some may be more tolerant to the nematode than others. Until resistant cultivars are available to farmers, appropriate crop rotations and cultivation techniques are likely to be the best strategy available. In the future, resistant varieties, combined with rotations, will offer the best approach to limiting damage caused by *P. neglectus*.

In view of the above information, different cereal and legume varieties were tested to determine the population of *P. neglectus* they were capable of supporting. Chickpea (*Cicer arietinum*; variety Amethyst) was studied in more detail, to illustrate the damage caused to roots by *P. neglectus*.

12.2 METHODS

Pratylenchus neglectus Populations in Cereal and Legume Varieties

Soil infested with *P. neglectus* was collected from below cultivation depth at the Cambrai (Figure 3.1) field site, and elutriated (as described in the General Methods) to determine the number of *P. neglectus* present. Cereal and legume varieties tested were planted in Hygienic Lily® plastic cups, containing 650 g of this soil, with an initial population of 500 *P. neglectus* per pot.

Five seeds were planted in five replicate pots for each variety. Pots were placed, in the glasshouse, in a waterbath maintained at 15±2°C. At the end of six weeks, soil was washed from the roots under running tapwater, and roots were misted (as described in the General Methods) to extract *P. neglectus* from the roots. The number of nematodes per plant was counted.

Pratylenchus neglectus Populations in Wheat Varieties Tolerant to P. thornei

T. aestivum varieties and selections tolerant to *P. thornei* were obtained from the Queensland Wheat Research Institute, and planted in pots of Murray Mallee field soil infested with *P. neglectus*, as described above. After six weeks, numbers of *P. neglectus* in the roots were determined and compared with those in Gatcher (intolerant to *P. thornei*) and the South Australian variety Machete.

Symptom Development on Amethyst Chickpea

Amethyst chickpea was inoculated with *P. neglectus* to investigate the development of symptoms on the roots. Surface-sterilised, pre-germinated seed was planted, to a depth of 1.0 cm, in tubes (125.0 mm long, 32.0 mm diameter) of moist,

steam-sterilised Murray Mallee field soil. Four tubes were each inoculated with 1.0 ml of SDW containing 1500 *P. neglectus*. The same amount of SDW was added to each of four control tubes. These tubes were supported in a tray of sand, placed in the glasshouse, and watered with distilled water as required. Water was added to the sand, to reduce the risk of nematodes being washed through the tubes.

At the end of one and three weeks, two inoculated and two uninoculated tubes were washed out under running tapwater. Symptoms on the roots were described, and roots were then fixed in FAA (as described in the General Methods). After at least 24 hours in the fixative, transverse or longitudinal sections of root were cut and stained in lactophenol-cotton blue for three to five minutes, then cleared in fresh lactophenol. These sections were investigated microscopically.

Effect of Pratylenchus neglectus on Emergence of Machete Wheat and Amethyst Chickpea

Surface-sterilised seed of Machete wheat and Amethyst chickpea was sown in moist, steam-sterilised Murray Mallee field soil in a polystyrene seedling tray. Individual compartments of the tray were 5.0 cm deep, with 3.0 cm square sides at the top tapering to 1.0 cm at the bottom. A single seed was sown, to a depth of 1.0 cm, in each compartment.

Four replicates of each species were inoculated with *P. neglectus* at a range of inoculum densities: 0, 100, 500, 800, 1000 and 1500 nematodes/plant. These nematodes had been extracted, by misting, from roots of wheat plants previously grown in pots of infested field soil. The nematodes were not aseptic, but had been washed several times in SDW. One millilitre of the appropriate nematode concentration, in SDW, was added to the soil directly above the seed, immediately following sowing.

The seedling tray was placed on the laboratory bench, at room temperature, to receive diffuse sunlight, and was watered with distilled water as required. The time of seedling emergence was recorded, and soil was washed from the roots under running tapwater at the end of twelve days. Symptoms on the roots were then observed.

12.3 RESULTS

Pratylenchus neglectus Populations in Cereal and Legume Varieties

All cereal and legume varieties tested were infected with *P. neglectus*, although there were differences in infection levels between species and within species (Table 12.1). Amethyst chickpea supported the highest number of *P. neglectus* recorded, and this was significantly ($P<0.05$) greater than that of any other plant species or variety tested. Gungurru lupin and Jemalong medic were infected with the fewest nematodes. Warrah lupin, however, was infected with more than four times the number of *P. neglectus* that Gungurru supported, and Yorrel nearly seven times the number on Gungurru, but these differences were not statistically significant. Vetch was infected with high numbers of *P. neglectus*, although Languedoc supported more than twice the population of Namoi, and the difference between these vetch species was significant ($P<0.05$). Some medics (Parabinga, Sephi, Parragio) were infected with moderate levels of *P. neglectus,* but others (Harbinger, Jemalong) supported a significantly ($P<0.05$) lower population of nematodes. The strand medic (Harbinger) was infected significantly ($P<0.05$) less than all barrel medics, except Jemalong.

There was no significant difference between varieties of *Triticum aestivum*, but Machete and Halberd were infected with more nematodes than the other varieties (Table 12.1). Spear wheat supported lower *P. neglectus* populations than the other varieties of *T. aestivum* tested. The roots of Galleon barley contained more *P. neglectus* than did all other cereal varieties, except Machete and Halberd wheat, but of the cereals, this was only significantly ($P<0.05$) more than durum variety Beliouni. Numbers on the rye variety were not significantly different from those recorded on other cereals, and were similar to those on the *T. aestivum* varieties. Echidna oats contained fewer *P. neglectus* than did *T. aestivum* varieties, but this was not significantly different from infection levels on other cereals. There was no significant difference in nematode numbers between the two triticale varieties, although they were lower than all cereals but the durum wheats. The durum varieties tested were infected with fewer nematodes than the other cereals, with variety Ruff infected the least and Medeah infected more than the other durums.

TABLE 12.1: Numbers of *Pratylenchus neglectus* extracted from the roots of six week old cereal and legume varieties grown in pots of infested field soil. (Values are the mean of five replicates for each variety).

Species	Variety	Nematodes/Plant
Chickpea (*Cicer arietinum*)	Amethyst	1734.0
Vetch (*Vicia sativa*)	Languedoc	948.8
Vetch (*V. villosa*)	Namoi	456.8
Lupin (*Lupinus angustifolius*)	Yorrel	150.0
	Warrah	97.7
	Gungurru	22.3
Strand Medic (*Medicago littoralis*)	Harbinger	47.9
Barrel Medic (*M. truncatula*)	Parabinga	405.7
	Sephi	398.0
	Parragio	361.0
	Jemalong	25.2
Barley (*Hordeum vulgare*)	Galleon	362.0
Oats (*Avena sativa*)	Echidna	114.3
Rye (*Secale cereale*)	S.A.	255.4
Triticale (*X Triticosecale*)	Tahara	107.1
	Currency	92.1
Bread Wheat (*Triticum aestivum*)	Halberd	366.9
	Machete	365.6
	Bindawarra	313.7
	Tatiara	307.0
	Aroona	299.4
	Schomburgk	283.1
	Molineux	270.9
	Kite	242.5
	Warigal	231.0
	Oxley	177.2
	Spear	113.4
Durum Wheat (*T. turgidum* var. *durum*)	Medeah	126.3
	Smooth Sbei	94.1
	Beliouni	80.1
	Senatore Cappelli	56.4
	Ruff	50.0
LSD (0.05)		309.0

However, this was a selected group of durums that had shown better root growth in the field, and the reaction of all durum varieties cannot be inferred from these results.

Plant density had a considerable influence on nematode numbers in roots. Although, in the experiments reported here, each pot contained five plants, a preliminary experiment (using the same soil) showed that roots were infected with many more *P. neglectus* when pots contained only one plant. With one plant per pot, 648 *P. neglectus* were extracted from durum variety Medeah after six weeks, and 676 from *T. aestivum* variety Machete. However, with five plants per pot, roots of Medeah and Machete contained 126 and 366 *P. neglectus*, respectively.

Pratylenchus neglectus Populations in Wheat Varieties Tolerant to P. thornei

Potam 70 (of Mexican origin) contained significantly ($P<0.05$) more *P. neglectus* than other varieties or selections tested (Table 12.2), although it is tolerant to *P. thornei*. There were no significant differences between other varieties, but all were infected with more *P. neglectus* than the South Australian variety Machete. Gatcher (intolerant to *P. thornei*) was not infected with any more *P. neglectus* than the others tested. In particular, infection of Gatcher was not significantly different from that of the *P. thornei* tolerant Gatcher selection (Gatcher 50A).

Symptom Development on Amethyst Chickpea

After one week, numerous dark brown, striate lesions had developed on the more mature sections of the main root axis (in the region within 2.0 - 3.0 cm below the seed) of inoculated seedlings. A few small (< 1.0 mm), light brown lesions had begun to develop on the younger tissues of the main root axis. Nematodes were not detected in the undamaged portions of the root. *P. neglectus*, but no eggs, were seen in the lesioned areas.

In transverse section, lesions could be seen through the epidermis, extending at least two to three cells into the cortex. Damaged cells were disorganised and distorted. Nematodes were present in these necrotic areas, although they had not penetrated the root very deeply, and were usually in the outer few layers of cortical cells. The nematodes

were often in groups, but some occurred singly, within or close to necrotic areas.

TABLE 12.2: Numbers of *Pratylenchus neglectus* extracted from the roots of six week old wheat varieties and selections grown in pots of infested field soil. Gatcher is intolerant to *P. thornei,* Machete is a South Australian cultivar, and others are tolerant to *P. thornei.* (Values are the mean of five replicates for each variety or selection).

Wheat Variety or Selection	Number of *P. neglectus*/Plant
Potam 70	1094.0
QT 4118	577.2
Gatcher 50A	559.7
Mr Sim	513.0
Funello	494.0
Autonomia	472.2
Funotto	421.3
Condor * Potam	312.0
Uruguay	211.3
Gatcher (intolerant to *P. thornei*)	339.0
Machete (South Australia)	210.9
LSD (0.05)	415.6

Roots of uninoculated plants were not lesioned or discoloured at either one or three weeks (Plates 12.1A, 12.2A). The actual number of *P. neglectus* in roots of inoculated plants was not determined at the end of one or three weeks, because all roots were fixed and cut into sections to investigate symptoms and to determine the association of these nematodes with the damage observed.

After three weeks, roots of inoculated seedlings exhibited dark brown, striate lesions up to 4.0 mm in length (Plate 12.1A). These were most severe on the more mature tissues within 2.0 - 3.0 cm below the seed. Lesions had also begun to develop on the younger tissues approximately 4.0 cm from the tip of the main root axis. In transverse section, nematodes could be seen associated with necrotic areas, although necrotic

PLATE 12.1: Three week old Amethyst chickpea seedlings that had been inoculated with 1500 *Pratylenchus neglectus*.

A. Numerous dark brown, striate lesions had formed on the more mature sections of the main root axis of inoculated (left) seedlings. Small lesions (➡) had also developed on the lateral roots in this region. The uninoculated control is on the right.

B. Seedlings inoculated with *P. neglectus* (right) produced similar numbers of laterals to the uninoculated (left) controls, but *P. neglectus* drastically reduced the length of these lateral roots.

Plate 12.1

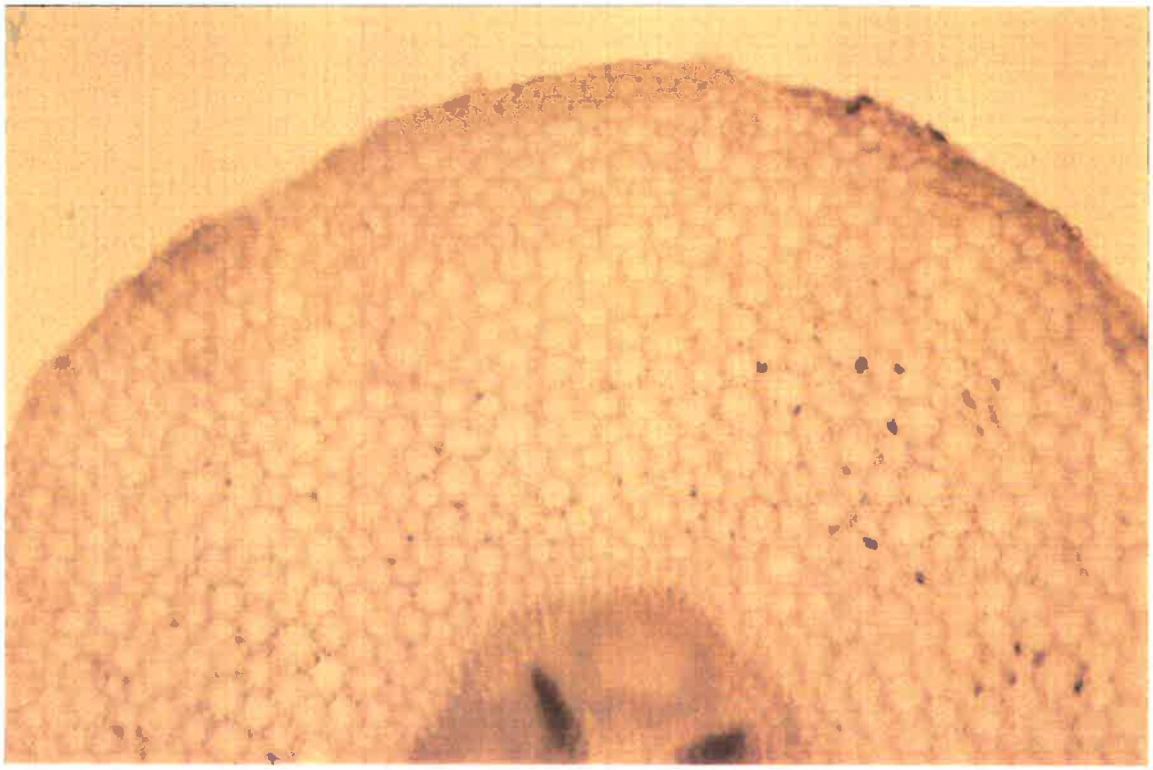
A

B

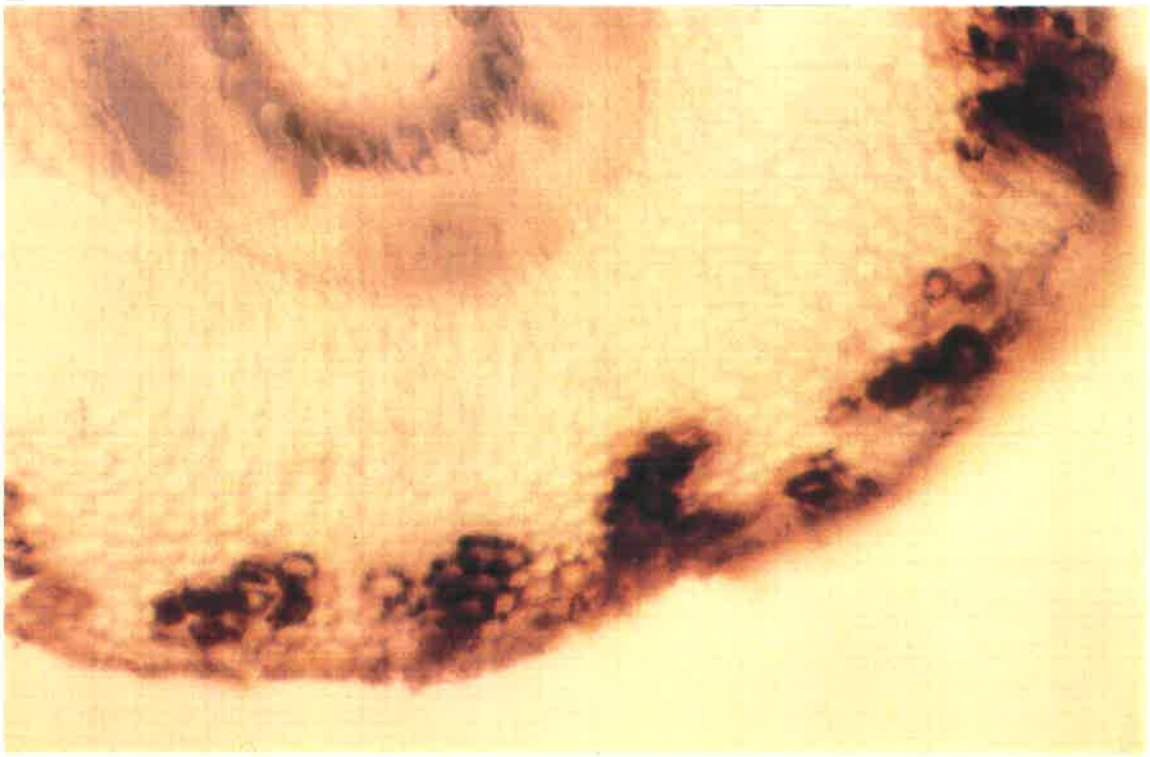
PLATE 12.2: Transverse sections of Amethyst chickpea roots from uninoculated controls (**A**; x100 magnification) or seedlings inoculated with *Pratylenchus neglectus* (**B**; x200 magnification). The necrotic areas in the cortex of inoculated roots occurred around the circumference of the whole root. Externally, this root was covered with dark brown, striate lesions (Plate 12.1A).

Plate 12.2

A



B



regions did not always contain nematodes. Several *P. neglectus* were present in most lesions, and one large lesion contained at least seven of these nematodes (Plate 12.3). The lesions had not developed to a much greater depth in the cortex, but covered a larger proportion of the root surface than at one week (Plate 12.2). The majority of nematodes were within cortical cells, but some were seen in the epidermis.

Lateral roots were also lesioned by three weeks (Plate 12.1A), and were poorly developed. Based on visual assessment of the roots, numbers of laterals were similar on inoculated and uninoculated plants, but inoculation with *P. neglectus* had drastically reduced the length of lateral roots (Plate 12.1B). The laterals on control plants were up to twice the length of those on inoculated plants. In longitudinal section, nematodes (Plate 12.4) and eggs could be seen within lesions on the lateral roots. No *P. neglectus* were detected in the undamaged portions of these roots. Tissues were also lesioned and discoloured in the region where laterals emerged from the main root axis. Some nematodes and eggs were detected in cortical cells in this area.

Effect of Pratylenchus neglectus on Emergence of Machete Wheat and Amethyst Chickpea

Emergence of Machete wheat was significantly ($P<0.05$) delayed by inoculation with 1000 *P. neglectus* (Table 12.3). Eight hundred and 1500 *P. neglectus* had no significant effect on Machete, although these inoculum levels did delay emergence by one day.

No level of inoculum significantly affected emergence of Amethyst chickpea (Table 12.3). However, as with wheat, 1000 *P. neglectus* did delay emergence, but only by one day.

After twelve days, roots of Machete were not visibly damaged. Amethyst roots displayed dark brown, striate lesions (as described elsewhere in this Chapter). The root growth of both species had been restricted due to the small volume of the seedling tray compartments.

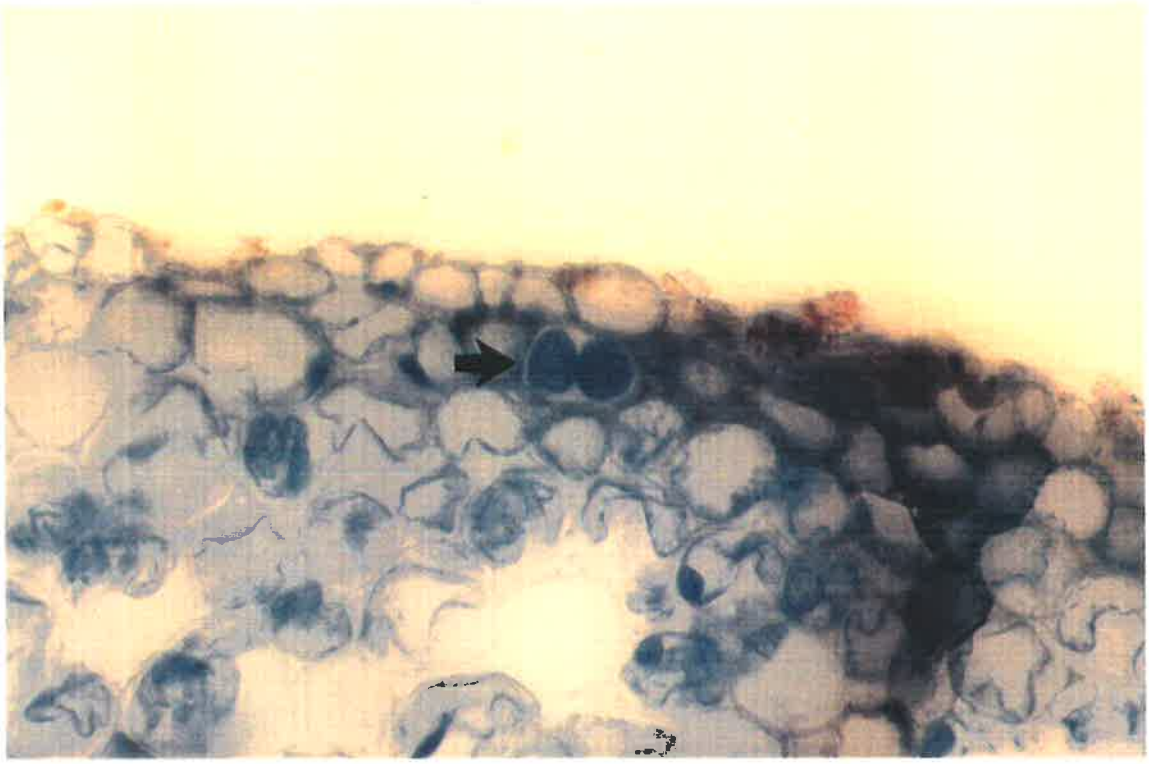
PLATE 12.3: Amethyst chickpea roots that had been inoculated with *Pratylenchus neglectus* were fixed, cut into transverse sections, and stained in lactophenol-cotton blue. Nematodes within the root were cut transversely, and appear as dark blue circles within cortical cells.

A. Two *P. neglectus* (➡) within cortical cells adjacent to necrotic tissue (darker cells to the right of the nematodes) (x400 magnification).

B. Seven (possibly eight) *P. neglectus* (➡) within a large, necrotic, cortical lesion (x400 magnification).

Plate 12.3

A



B

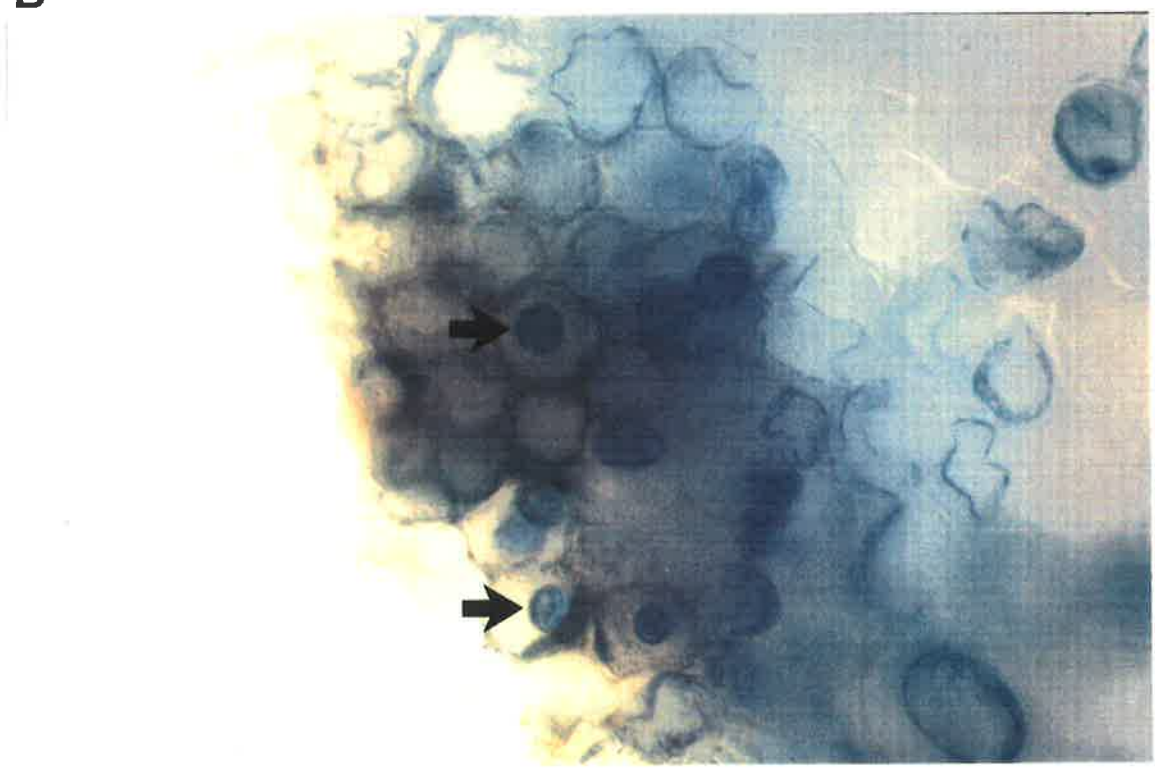


PLATE 12.4: Longitudinal section of lesioned lateral root of Amethyst chickpea that had been inoculated with *Pratylenchus neglectus* (x200 magnification). An individual nematode (➡) can be seen within the lesioned area (brown cells).

Plate 12.4

TABLE 12.3: The effect of *Pratylenchus neglectus* (nematodes/plant) on emergence (days) of Machete wheat and Amethyst chickpea seedlings. (Values are the mean of four replicates at each inoculum level).

Inoculum (nematodes/plant)	DAYS TO EMERGENCE	
	Machete	Amethyst
0	6.3	8.0
100	6.5	8.0
500	6.0	8.5
800	7.5	7.3
1000	8.5	9.0
1500	7.5	7.0
LSD (0.05)	2.1	2.8

12.3.1 Summary of Results

Assessment of cereal and legume varieties showed that:

(1) All cereal and legume varieties tested were infected with *P. neglectus*, but there were differences in population levels between species and within species.

(2) Legume species covered a wider range of infection levels than the cereals. Amethyst chickpea was infected with the highest number of *P. neglectus*, with Gungurru lupin and some medic varieties infected with the least nematodes.

(3) Varieties tested may be ranked as follows:

Very high	Amethyst chickpea
	Languedoc vetch
High	Namoi vetch
	Machete and Halberd wheat
	Galleon barley
	most barrel medics
Moderate	S.A. rye
	the majority of *T. aestivum* varieties tested
Low	Yorrel and Warrah lupin

	the durum wheat genotypes tested
	Echidna oats
	Tahara and Currency triticale
	Spear wheat
Very Low	Harbinger strand medic
	Jemalong barrel medic
	Gungurru lupin

(4) Distinct lesions were seen on the roots of Amethyst chickpea inoculated with *P. neglectus*. Dark brown, striate lesions were visible on more mature areas of the root after one week, and also on younger portions and laterals by three weeks. Nematodes, usually in groups, were present in cortical lesions. A few nematodes were also seen within epidermal cells. Lateral roots were drastically reduced in length. *P. neglectus* and eggs were present in lesions on lateral roots, and at the junction of laterals with the main root axis.

(5) One thousand *P. neglectus* significantly ($P<0.05$) delayed emergence of Machete wheat seedlings.

12.4 DISCUSSION

Although all cereal and legume varieties tested were infected with *P. neglectus*, there were differences in infection levels between and within species (Table 12.1). Other workers have also reported differences in *Pratylenchus* spp. populations on various crops (Oostenbrink, 1961; Greco *et al.*, 1988). These differences probably indicate a range in resistance to the nematode, but the effect these levels of infection had on the plants was not assessed. Perhaps one species or variety infected with over 900 *P. neglectus* may be quite healthy, whereas another supporting 200 - 300 nematodes may be severely reduced in vigor, and consequently yield, especially in conjunction with fungal infection of the roots. Theoretically, threshold populations at which *P. neglectus* deleteriously affect plants could be determined for each crop by regression analysis of yield or level of root damage against *P. neglectus* numbers in the roots. Olthof (1990) reported that low initial

densities of *P. neglectus* can cause yield losses in potato. In 1986, 115 nematodes per kilogram of soil depressed yield by 12%, and in the following year, 186 *P. neglectus* per kilogram of soil reduced potato yield by 22%. *P. neglectus* numbers at Murray Mallee field sites examined in 1989 were up to 780/kg of soil. By Olthof's (1990) standards, this would be devastating to potato plants, but the effect on the yield of wheat and other crops is yet to be demonstrated. Nevertheless, different crops, and crop varieties, will have varying effects on subsequent numbers of nematodes in the soil.

Crops following Amethyst chickpea, and possibly other chickpea varieties, will be exposed to high populations of *P. neglectus*. Thompson (1987) reported that chickpea was comparable to wheat as a host of *P. thornei*, and caused nematode populations in the soil to build-up. In fact, information from farmers in the Mid-North of South Australia indicates that wheat crops following chickpeas sometimes lack vigor and yield poorly. Single paddocks planted to different crops can provide useful information on the effects of rotation on crop growth. One farmer in the South East of South Australia noted that wheat planted in a paddock previously sown with chickpeas and faba beans (*Vicia faba*) performed better where the beans had grown than where the chickpeas had been grown. These observations may indicate that wheat was adversely affected by high nematode populations in the soil following chickpeas. However, chickpea varieties vary in their reaction to *P. thornei* (Thompson, 1987; Greco *et al.*, 1988), and probably behave similarly to *P. neglectus*. Faba beans, which were not tested in these experiments, may harbour lower numbers of *P. neglectus* than chickpeas, although they have been shown to contain very high *P. thornei* populations in Syria (Greco *et al.*, 1988).

These tests, albeit on only a few of the available varieties of each species, indicate that rotations including lupins or, perhaps, some medic varieties will probably limit *P. neglectus* numbers in the soil, thus reducing the damage caused to subsequent cereal crops. Annual medics, however, can be infected with very high populations of *P. thornei* (Greco *et al.*, 1988). Oats and triticale may be better than some of the other cereals, although the spread of the stem nematode (*Ditylenchus dipsaci*) in South Australia may limit the use of oats in such rotations. Townshend (1989) found that all oat varieties tested were infected with *P. penetrans* and *P. neglectus,* but varieties did differ in their

reaction to the nematodes, with one variety being more resistant than the others tested.

The durum wheats tested in this experiment were infected less than the other cereals, but are not widely grown in South Australia, although this may change in the future. Rye and barley varieties tested were no better than the other cereals, and limited bioassay of a paddock at Cambrai infested with *P. neglectus* indicated that Machete and Warigal wheat were infected with more *P. neglectus* after Galleon barley than after Halberd wheat. However, in Queensland, Thompson (1987) suggests that barley, along with sorghum and sunflower, is a useful rotation crop in areas with high *P. thornei* populations. Roots of *T. aestivum* varieties contained similar numbers of *P. neglectus*, but Machete seems to support higher populations than the other varieties tested. Languedoc vetch (*Vicia sativa*) and Namoi vetch (*V. villosa*) supported the highest *P. neglectus* populations, after Amethyst chickpea. Greco *et al.* (1988) also found that vetch (*V. sativa*) was infected with very high numbers of *P. thornei*.

No plants tested were free of nematodes, but Greco *et al.* (1988) detected several species in Syria that were not infected with *P. thornei*: turnip, radish (*Raphanus sativus*), parsley and kumboz (*Cannabis sativa*). Unfortunately, these are unsuitable for South Australian cereal cropping systems (with one being a "prohibited plant"), and their reaction to *P. neglectus* would possibly be different from that recorded for *P. thornei*.

A range of responses to *P. neglectus* exists in the material tested, which indicates that breeding for resistant or tolerant local varieties will be possible. In Mexico, wheat varieties displayed a range of reactions to *P. thornei* (Van Gundy *et al.*, 1974), and O'Brien (1983) reported similar reactions to *P. thornei* in Australian wheat varieties.

Little information is available on the relative populations of *P. neglectus* in different crops. More work in this area has been done with *P. thornei* and *P. penetrans*, but is unlikely to be directly applicable to *P. neglectus*. Pot trials with Queensland wheat varieties and selections resistant or tolerant to *P. thornei* indicated that these had no resistance to *P. neglectus*, and all supported similar or higher numbers of *P. neglectus* than the local variety Machete (Table 12.2).

The plants in this experiment were grown in pots of untreated field soil that probably contained some fungi and other organisms, as well as the nematodes, even

though it was collected from below cultivation depth, where fungal frequencies are lower and *Pratylenchus* populations higher than in the cultivated layer of soil. Roots would have been subjected to at least some fungal infection, which is similar to the situation in the field. These experiments would then have given a reasonably realistic comparison to conditions in the field, where fungi and nematodes both influence the host, as well as each other. However, in pots, root:soil ratios are usually higher than those found in the field (Scholte and s'Jacob, 1989). In fact, the roots of field-grown plants only explore a small volume of the available soil (Russell, 1977).

Plant density in pots has a great effect on the extent of nematode infection. When only one plant was sown in pots of field soil, *T. aestivum* variety Machete was infected with 676 *P. neglectus*, and durum variety Medeah with 648. However, with five plants per pot, the *P. neglectus* densities in roots were 366 and 126, respectively. Plant density in the field will also influence nematode populations in the roots. Medics, under field conditions, would be sown at higher densities than are cereals. Although, on an individual plant basis, medics contain fewer *P. neglectus* within the roots, total populations under medics and cereals in the field may not retain the same relationship. To represent more accurately plant and root densities in the field, plants in future could be sown in large boxes of infested field soil in the glasshouse.

Before species reaction to *P. neglectus* can be determined accurately, and consequently the effect of these on nematode numbers in the soil, many more varieties of each species need to be tested thoroughly. Furthermore, research is needed to establish whether the relationship between nematode populations and species/varieties in pots reflects that in the field. Weeds could also be investigated to assess population densities in the roots. *P. neglectus* has a very wide host range, and would undoubtedly infect many weeds that occur in crops and pastures. One sample of barley grass (*Hordeum leporinum*) collected from a paddock at Roseworthy Agricultural College in 1989 supported an extremely high population of this nematode.

Amethyst chickpea seems to provide a good model to investigate nematode infection of roots and the development of symptoms. *P. neglectus* readily infects Amethyst, causing considerable damage to cortical and epidermal cells in only a few

weeks. Lesions were produced on the roots within one week, and lateral root production was inhibited by the end of three weeks. Inoculation of this chickpea showed that the development of lesions is associated with nematode infection, resulting in large necrotic areas within the root cortex. Narrow, elongate lesions, as seen on the chickpea roots, are characteristic of infection with *Pratylenchus* spp. (Dropkin, 1989). However, numbers of nematodes within these roots may actually decrease as the plant matures, resulting in low numbers being detected in the roots of field-grown plants by the end of the growing season. If root cells are destroyed by the large population of nematodes infecting seedlings, this may restrict feeding and multiplication of *P. neglectus* at a later time.

Pratylenchus spp. do not feed on vascular tissues, and Zunke (1990) found that *P. penetrans* did not invade the stele of roots. The vascular tissue of Amethyst was not visibly damaged, but if fungi had also been present, the large cortical lesions would have provided them with easier access to the central stelar region of the roots.

P. neglectus were not always seen within necrotic areas, but nematodes were not observed in adjacent undamaged cortical cells. *Pratylenchus* spp. vacate damaged tissues (Dropkin, 1989), and some of the thin, transverse sections may have been taken in areas not containing nematodes, especially as lesions on the root surface were up to 4.0 mm long. Nematodes usually occurred in groups, which is a phenomenon documented for *Pratylenchus* spp. (Baxter and Blake, 1968), although some lesions contained only one or two individuals. Presumably, some lesions were caused by several individuals, while others were the work of single nematodes. By three weeks, the lesions had begun to coalesce, in some cases almost girdling the root.

Cells within necrotic regions were distorted. This is caused by the nematodes feeding, but they also cause considerable cellular damage by their movements within the root (Zunke, 1990).

The nematodes first infected the main root axes, later damaging the lateral roots. Bhatt (1986) reported that *P. thornei* preferred the more mature areas of chickpea roots, and the nematodes did not infect at or near the root apex. In the work reported here, lateral roots were lesioned, and their length severely retarded. This accords with observations on the effects of *P. neglectus* on the growth of wheat lateral roots (Chapters

10 and 11). The area where lateral roots emerged from the main root axes were discoloured, and some nematodes were detected in this region. Corbett (1972) reported that *P. fallax* were attracted to the junction of laterals with the main root axes of wheat, and nematodes could be seen in the cells around the base of developing lateral roots. Zunke (1990) found that *P. penetrans* also behaved this way.

Pitcher *et al.* (1960) suggest that the extent of root damage caused by root lesion nematodes depends on the presence of specific plant constituents which, when acted upon by enzymes produced by the nematode, give rise to products toxic to host cells. Tissues that become rapidly discoloured often contain high concentrations of phenolic substances. *P. penetrans* breaks down amygdalin, probably by means of a β-glucosidase enzyme, releasing phytotoxins (Mountain and Patrick, 1959). Not all plants contain amygdalin, but β-glucosidase is active against other phenolic substances (Pitcher *et al.*, 1960). Acedo and Rohde (1971) showed that the browning of cabbage roots, caused by *P. penetrans*, was associated with the accumulation of polyphenols. Chickpea roots may contain higher concentrations of these chemicals than are found in wheat roots, thus showing more visible signs of nematode damage (in the form of striate lesions on the root surface, and groups of necrotic cells within the cortex). Although cortical cells of wheat roots become disorganised due to nematode infection (Corbett, 1972), they do not develop lesions due to invasion by *P. neglectus* like those seen on chickpea roots. Differences in chemical constituents may explain the observation of Dropkin (1989) that *Pratylenchus* spp. do not cause distinct lesions on the roots of all hosts.

Pratylenchus spp. can interfere with nodulation of legume roots, but this was not assessed, as plants were only three weeks old, and growing in sterile soil. Townshend (1984) found that *P. penetrans* and *P. neglectus* restricted nodulation on lucerne (alfalfa; *Medicago sativa*), and Bhatt (1986) reported that *P. thornei* reduced nodulation of chickpea roots. *P. neglectus* significantly delayed emergence of Machete wheat seedlings, but had no effect on Amethyst chickpea under the conditions of this experiment (Table 12.3).

Chickpeas are readily infected by *P. neglectus*, resulting in the development of distinct symptoms on and within the roots, that can be directly attributed to the presence

of the nematode. This species is therefore an excellent model to use in studying the epidemiology and aetiology of disease caused by *P. neglectus*.

CHAPTER 13

GENERAL DISCUSSION AND CONCLUSIONS

The problem of root rots in wheat is complex. Many organisms are present in rotted roots, but the presence of a particular parasite does not necessarily indicate that it is responsible for the observed root damage. Koch's postulates still need to be proven, to associate the organisms identified with the appearance and severity of symptoms. This is, however, a difficult task to perform successfully, especially as several fungi that may be only "minor" pathogens in their own right seem to contribute to root damage in the field.

These difficulties are compounded by the inherent variability of such inoculation experiments, which obscures the significance of results. Until the sources of this variation are identified and controlled, the role of different organisms in root rotting cannot be defined with certainty. Even then, these organisms will not behave identically in pots and in the field, and one organism alone is unlikely to be responsible for the root rot condition investigated in this study. Climatic, edaphic and biotic conditions differ markedly between pots and the field. Furthermore, plants may need to be subjected to some degree of water and nutrient stress before the severity of symptoms observed under field conditions is reproduced.

Gaeumannomyces graminis, *Rhizoctonia solani*, *Bipolaris sorokiniana* and *Fusarium graminearum* are recognised as "major" pathogens of wheat, and root damage is often attributed to one of these fungi. However, these were isolated rarely, and there was little evidence to suggest that they significantly contributed to the root rotting. During the course of this study, *G. graminis* was isolated from only 3 - 5% of root samples, and *R. solani* from only 5% of these roots. The relationship between *B. sorokiniana* and poor plant growth was tenuous (Chapter 4), and this species infected only a small proportion (2 - 11%) of subcrown internodes, without significantly contributing to root damage. *Fusarium* spp. infected a large proportion of the roots examined (36 - 95%), but *F. graminearum* was infrequently detected in root tissues. Other species were encountered more frequently than were the so-called "major" pathogens. *Microdochium bolleyi*

infected 13 - 26% of roots tested, and *Pythium* spp. colonised, on average, 79% of roots sampled.

Under controlled conditions, *M. bolleyi* appeared to afford some control against *B. sorokiniana* (Chapter 8), and when acting alone, *M. bolleyi* caused negligible root damage (Chapter 7). However, in the field, combined with other root infecting organisms, *M. bolleyi* may contribute to root damage. This species is widespread, occurs at high levels in the soil, and readily infects a large proportion of root and subcrown internode tissue.

Pratylenchus neglectus was invariably associated with diseased roots sampled from Murray Mallee field sites in 1989 (Chapters 9 and 10), but the nematode alone caused little visible damage to wheat roots (Chapter 11). In conjunction with fungi, however, this nematode may significantly contribute to root damage. Initial evidence that *P. neglectus* plus *Pythium* causes more severe root damage than either pathogen alone was promising (Chapter 11). Inoculation with both organisms more closely reproduced the symptoms that were observed in the field than did any other inoculation treatment tested. The full range and severity of symptoms, however, was still not attained. Other species of fungi are undoubtedly involved in causing the damage, but this hypothesis requires further investigation, using nematodes that have been cultured under aseptic conditions.

Fusarium spp. (other than *F. graminearum*), *M. bolleyi* and *Pythium* spp. are the obvious candidates to test in conjunction with the nematode (Chapters 10 and 11). Bacteria may also contribute to the rotting of roots already damaged by nematodes and fungi. Different combinations of fungi and bacteria need to be tested in conjunction with *P. neglectus*, to reproduce the severity of root damage observed in the field, before the incitants of such disease can be accurately defined. Nevertheless, it seems that roots need to be colonised by both *P. neglectus* and fungi for full disease expression, together with the conditions inducing plant stress in the field. The rotting and decortication of crown roots (Chapter 9), and the inhibition of lateral root growth (Chapters 6 and 10), appears to be caused by such a combination of pathogens.

Some of the symptoms observed were similar to those usually attributed to *R.*

solani. However, this species was isolated infrequently, and significantly decreased in occurrence as the severity of root damage increased (Chapter 9). *M. bolleyi* (Chapter 7) or *P. neglectus* plus *Pythium* (Chapter 11) produced symptoms that could be mistaken for those ascribed to *R. solani*, and many other fungi were isolated from roots with such symptoms. It is thus imperative that disease is not diagnosed from the appearance of symptoms alone, and that an active role in root damage is not inferred from the mere presence of a parasite within the roots. Organisms present within rotted tissues must be isolated and identified, then associated with the symptoms that occur during the course of the growing season. The problem of accurate disease diagnosis is compounded by the fact that numbers of *P. neglectus* within roots decline as root rot increases in severity.

This study has revealed many areas that warrant further investigation. In particular, the combined effect of *P. neglectus* and various fungi on plant growth and the development of symptoms requires additional research. However, experimental conditions need some modification if this is to prove successful. Better methods of inoculating plants with *P. neglectus*, and then extracting the nematodes from roots, must be sought. Field studies of this nematode require more accurate methods of sampling roots and soil to determine population levels over the growing season.

The effects of the described root damage need to be quantified in terms of reductions in plant growth and grain yield. As several organisms are involved, this would be difficult to demonstrate in field trials. The relationship of symptoms and plant growth with inoculum densities of fungi and nematodes could be quantified in pots, but such results would not be strictly applicable to the situation in the field.

Screening wheat varieties to detect sources of resistance or tolerance to the nematode could be incorporated into wheat breeding programs in South Australia. However, if these measures are to be successful, the biology of *P. neglectus* and the aetiology of disease under South Australian climatic and cultural conditions will need to be elucidated.

Since wheat roots in the field are not colonised by only one organism, it is reasonable to conclude that root damage results from the combined activities of several pathogens. It is thus essential to continue to study relationships between such organisms

if root disease is to be understood and controlled. The hypothesis that root damage in South Australian wheat crops is caused by *P. neglectus* plus associated fungi and bacteria offers an explanation more tenable than those postulated in the past.

APPENDIX A

Transformed means and LSD (0.05) values for isolation frequencies of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) corresponding to Figures 8.1 - 8.7.

Plots on fumigated (F) and non-fumigated (NF) areas at Sanderston and Mannum were inoculated with *Microdochium bolleyi* (Mb), *Bipolaris sorokiniana* (Bs), both fungi (MbBs) or neither species. Values are pooled for all wheat varieties.

TABLE A.1: Isolations from seminal roots of wheat in June, 1988 (mean of four replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.1.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	1.3	0.7	1.5	1.5
Bs-F	0.7	0.7	0.7	0.7
MbBs-F	0.7	0.7	0.7	0.7
F	1.3	0.7	0.7	1.5
Mb-NF	3.6	2.1	2.7	1.5
Bs-NF	1.8	2.1	4.1	2.6
MbBs-NF	1.8	1.0	2.0	0.7
NF	2.4	0.7	2.0	2.0
LSD (0.05)	1.5	1.5	1.5	1.9

TABLE A.2: Isolations from seminal roots of wheat in July, 1988 (mean of seven replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.2.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	2.7	0.7	1.9	0.7
Bs-F	1.9	0.7	0.7	0.7
MbBs-F	2.6	0.7	2.8	1.2
F	1.9	1.5	2.0	0.7
Mb-NF	3.9	1.4	2.7	1.7
Bs-NF	2.7	0.7	3.0	1.7
MbBs-NF	3.0	2.1	1.9	2.2
NF	1.3	0.7	2.8	1.5
LSD (0.05)	2.6	1.4	2.4	1.5

TABLE A.3: Isolations from subcrown internodes of wheat in July, 1988 (mean of nine replicates). Values are √(x+0.5) transformations. Refer to Figure 8.3.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	2.0	0.7	2.9	1.0
Bs-F	0.7	0.7	1.4	0.7
MbBs-F	2.6	1.1	3.6	0.7
F	1.9	1.1	1.4	0.7
Mb-NF	2.1	1.1	1.7	0.7
Bs-NF	0.7	0.7	2.6	1.0
MbBs-NF	2.4	0.7	2.1	1.0
NF	2.4	0.7	1.6	1.0
LSD (0.05)	2.1	0.6	1.6	0.6

TABLE A.4: Isolations from seminal roots of wheat in August, 1988 (mean of eight replicates). Values are √(x+0.5) transformations. Refer to Figure 8.4.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	1.8	0.7	1.3	2.2
Bs-F	1.1	0.7	0.7	1.3
MbBs-F	1.1	0.7	2.4	1.3
F	3.1	0.7	1.9	0.7
Mb-NF	2.6	0.7	4.3	0.7
Bs-NF	2.0	0.7	3.4	2.9
MbBs-NF	3.2	0.7	3.3	3.0
NF	1.9	0.7	2.8	1.3
LSD (0.05)	2.0	-	2.3	1.7

TABLE A.5: Isolations from subcrown internodes of wheat in August, 1988 (mean of eight replicates). Values are arcsine √% transformations. Refer to Figure 8.5.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	34.1	9.1	22.8	9.1
Bs-F	17.7	10.6	13.0	11.0
MbBs-F	19.3	12.0	30.0	11.0
F	13.5	10.6	32.9	10.8
Mb-NF	18.0	9.1	29.4	15.8
Bs-NF	11.9	11.9	12.6	21.7
MbBs-NF	23.1	9.1	20.5	23.2
NF	13.4	9.1	24.7	18.2
LSD (0.05)	11.5	3.2	15.2	8.0

TABLE A.6: Isolations from crown roots of wheat in September, 1988 (mean of eight replicates). Values are arcsine √% transformations. Refer to Figure 8.6.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	44.0	10.0	38.5	11.8
Bs-F	27.4	13.5	18.4	15.3
MbBs-F	34.9	10.0	24.5	10.0
F	20.9	11.8	20.2	15.3
Mb-NF	33.7	15.3	29.1	15.3
Bs-NF	38.3	10.0	27.2	17.0
MbBs-NF	34.7	11.8	29.1	15.3
NF	31.7	10.0	28.6	11.8
LSD (0.05)	14.0	4.4	12.6	6.4

TABLE A.7: Isolations from subcrown internodes of wheat in September, 1988 (mean of eight replicates). Values are arcsine √% transformations. Refer to Figure 8.7.

	SANDERSTON		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb-F	33.8	12.4	37.6	9.1
Bs-F	20.5	18.4	20.2	17.3
MbBs-F	24.4	12.4	30.3	12.0
F	17.0	9.1	19.0	14.6
Mb-NF	20.0	11.0	14.3	16.8
Bs-NF	22.3	12.1	26.3	15.0
MbBs-NF	20.6	12.1	15.3	12.4
NF	13.7	10.8	18.2	12.4
LSD (0.05)	13.1	7.1	13.7	7.7

APPENDIX B

Transformed means and LSD (0.05) values for isolation frequencies of *Microdochium bolleyi* (Mb) and *Bipolaris sorokiniana* (Bs) corresponding to Figures 8.8 - 8.15.

Plots on fumigated (F) and non-fumigated (NF) areas at Caloote and Mannum were inoculated with low (Mb1) and high (Mb2) rates of *Microdochium bolleyi*, alone and in conjunction with *Bipolaris sorokiniana* (Bs). Control plots (F and NF) were uninoculated.

TABLE B.1: Isolations from seminal roots of Machete wheat in July, 1989 (mean of four replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.8.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	1.3	1.3	1.3	1.8
Mb2-F	0.7	1.3	1.3	1.3
Bs-F	0.7	0.7	0.7	0.7
Mb1Bs-F	1.8	0.7	0.7	0.7
Mb2Bs-F	1.8	2.7	2.3	0.7
F	1.3	1.3	2.1	1.3
Mb1-NF	3.6	1.3	3.6	0.7
Mb2-NF	1.3	0.7	3.2	0.7
Bs-NF	3.5	1.8	4.6	2.2
Mb1Bs-NF	2.4	0.7	2.7	1.3
Mb2Bs-NF	0.7	0.7	1.8	1.3
NF	3.2	0.7	1.8	1.3
LSD (0.05)	1.9	1.6	2.3	1.4

TABLE B.2: Isolations from seminal roots of Kite wheat in July, 1989 (mean of four replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.9.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	1.8	1.3	1.3	0.7
Mb2-F	0.7	0.7	1.3	0.7
Bs-F	0.7	0.7	1.3	0.7
Mb1Bs-F	1.3	0.7	0.7	1.3
Mb2Bs-F	1.3	1.8	0.7	0.7
F	1.9	0.7	1.3	0.7
Mb1-NF	1.3	1.3	2.7	1.3
Mb2-NF	1.3	0.7	2.7	1.9
Bs-NF	1.8	0.7	3.0	1.3
Mb1Bs-NF	1.8	1.3	4.3	0.7
Mb2Bs-NF	2.6	0.7	2.1	1.3
NF	2.1	0.7	4.1	0.7
LSD (0.05)	2.0	1.1	2.3	1.4

TABLE B.3: Isolations from subcrown internodes of Machete wheat in July, 1989 (mean of four replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.10.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	2.1	0.7	3.2	0.7
Mb2-F	1.4	0.7	3.5	0.7
Bs-F	1.8	0.7	4.5	2.1
Mb1Bs-F	0.7	1.4	3.5	0.7
Mb2Bs-F	1.8	2.7	2.6	0.7
F	1.5	0.7	2.6	0.7
Mb1-NF	2.5	0.7	4.1	0.7
Mb2-NF	2.1	0.7	5.0	0.7
Bs-NF	0.7	0.7	3.4	0.7
Mb1Bs-NF	4.6	0.7	5.7	1.5
Mb2Bs-NF	3.2	0.7	3.2	0.7
NF	1.4	0.7	3.0	0.7
LSD (0.05)	3.0	1.3	3.7	1.3

TABLE B.4: Isolations from subcrown internodes of Kite wheat in July, 1989 (mean of four replicates). Values are √(x+0.5) transformations. Refer to Figure 8.11.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	2.1	0.7	3.2	0.7
Mb2-F	1.4	1.4	3.2	0.7
Bs-F	2.1	0.7	2.1	0.7
Mb1Bs-F	3.0	0.7	2.2	1.4
Mb2Bs-F	2.2	0.7	1.4	1.4
F	1.5	0.7	1.4	0.7
Mb1-NF	3.8	0.7	1.4	0.7
Mb2-NF	1.4	1.8	2.8	0.7
Bs-NF	3.7	0.7	4.3	0.7
Mb1Bs-NF	0.7	1.4	2.1	0.7
Mb2Bs-NF	3.2	0.7	4.1	0.7
NF	3.2	0.7	2.5	1.4
LSD (0.05)	3.1	1.3	3.0	1.0

TABLE B.5: Isolations from seminal roots of Machete wheat in August, 1989 (mean of three replicates). Values are √(x+0.5) transformations. Refer to Figure 8.12.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	2.4	0.7	0.7	0.7
Mb2-F	0.7	0.7	0.7	1.9
Bs-F	3.0	0.7	0.7	0.7
Mb1Bs-F	3.0	0.7	1.9	0.7
Mb2Bs-F	3.6	0.7	2.4	0.7
F	0.7	0.7	0.7	0.7
Mb1-NF	1.9	0.7	1.9	3.0
Mb2-NF	2.4	0.7	3.0	1.9
Bs-NF	3.0	0.7	1.9	1.8
Mb1Bs-NF	4.4	2.4	0.7	0.7
Mb2Bs-NF	5.2	0.7	1.9	0.7
NF	1.9	1.9	1.9	0.7
LSD (0.05)	4.3	1.8	2.4	1.8

TABLE B.6: Isolations from seminal roots of Kite wheat in August, 1989 (mean of three replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.13.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	0.7	0.7	0.7	0.7
Mb2-F	4.0	0.7	0.7	0.7
Bs-F	0.7	0.7	1.9	0.7
Mb1Bs-F	0.7	0.7	1.9	0.7
Mb2Bs-F	0.7	3.2	0.7	0.7
F	1.8	0.7	1.9	0.7
Mb1-NF	5.3	0.7	3.0	0.7
Mb2-NF	2.8	0.7	3.6	0.7
Bs-NF	4.1	0.7	6.3	0.7
Mb1Bs-NF	3.6	1.9	3.6	0.7
Mb2Bs-NF	2.4	0.7	4.1	0.7
NF	4.7	0.7	0.7	0.7
LSD (0.05)	4.2	2.2	2.8	-

TABLE B.7: Isolations from subcrown internodes of Machete wheat in August, 1989 (mean of three replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.14.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	0.7	0.7	1.7	0.7
Mb2-F	0.7	0.7	0.7	0.7
Bs-F	0.7	0.7	1.7	1.7
Mb1Bs-F	2.5	0.7	0.7	0.7
Mb2Bs-F	1.7	0.7	1.7	0.7
F	0.7	0.7	1.7	0.7
Mb1-NF	0.7	1.7	2.6	0.7
Mb2-NF	1.7	0.7	2.2	2.2
Bs-NF	0.7	1.8	1.8	6.3
Mb1Bs-NF	2.2	0.7	3.8	1.7
Mb2Bs-NF	2.4	0.7	1.7	0.7
NF	2.0	0.7	1.7	3.1
LSD (0.05)	2.9	1.2	3.4	1.9

TABLE B.8: Isolations from subcrown internodes of Kite wheat in August, 1989 (mean of three replicates). Values are $\sqrt{(x+0.5)}$ transformations. Refer to Figure 8.15.

	CALOOTE		MANNUM	
Treatment	**Mb**	**Bs**	**Mb**	**Bs**
Mb1-F	2.5	0.7	1.7	0.7
Mb2-F	0.7	0.7	2.6	0.7
Bs-F	0.7	0.7	1.7	0.7
Mb1Bs-F	0.7	0.7	0.7	0.7
Mb2Bs-F	1.9	1.9	0.7	0.7
F	0.7	0.7	1.7	0.7
Mb1-NF	2.8	1.6	3.1	1.7
Mb2-NF	0.7	0.7	1.7	1.7
Bs-NF	3.5	0.7	3.1	1.9
Mb1Bs-NF	0.7	0.7	3.1	2.6
Mb2Bs-NF	2.8	0.7	2.5	0.7
NF	0.7	0.7	2.8	0.7
LSD (0.05)	3.4	1.0	3.3	1.5

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