Studies on common root rot and *Bipolaris sorokiniana* in wheat and barley in South Australia

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ABSTRACT

Surveys of South Australian wheat and barley crops for common root rot were conducted in 1988 and 1989. The disease was present throughout the state's cereal belt in many different environments. In 1988, the mean incidence of diseased plants in crops was 60% in wheat, and 77% in barley. The corresponding values in 1989 were 34% and 49%. Temperature during winter was probably an important determinant of annual variation in disease levels. Levels of common root rot in wheat crops corresponded with their varietal resistance ratings.

Progress of common root rot through the growing season was followed in Machete wheat at six field sites in 1987. Percentage of plants with lesions ranged from 0 to 60% 83 days after sowing, and from 37 to 95% after 175 days. The differences between sites indicated that other factors besides temperature were important in determining disease development rate and final levels.

Bipolaris sorokiniana, the putative common root rot pathogen, was isolated from 105 out of 115 wheat crops in 1988, with a mean isolation frequency from lesioned subcrown internodes of 42%. In Machete wheat in the field in 1989, *B. sorokiniana* was isolated from 9% of unlesioned subcrown internodes and 43% of diseased ones. Two isolates were tested for pathogenicity on wheat and barley in field soil with a low background inoculum density of *B. sorokiniana*. Lesions on the subcrown internode and roots, such as observed on field samples, were present in both inoculated and control treatments, but were more severe in the inoculated. *B. sorokiniana* was more frequently isolated from inoculated than control plants. There was a strong correlation ($r = 0.81$) between lesion severity and isolation frequency. Slightly diseased tissue gave rise to smaller colonies than did severely diseased tissue. Thus, through Koch's Postulates, *B. sorokiniana* was demonstrated to be the probable cause of most, if not all, of the common root rot observed in the field. In this work, a plating method ("water agar/DR70 sandwich") was developed for selective isolation of *B. sorokiniana*, using the selective medium of Dodman and Reinke (1982). The

sandwich gave improved selectivity, was highly efficient, allowed accurate quantitation of disease severity and tissue colonisation, and allowed the association of these two variates to be investigated.

Field experiments were conducted on resistance to common root rot in wheat and barley. In both crops, there was variability in disease severity among genotypes, and ratings were quite consistent between sites, although the range and consistency was much greater in wheat than in barley. Genotype*site interactions were observed, but may have been statistical artefacts, rather than true interactions. Wheat varieties covering a large proportion of the state's wheat area were rated susceptible. Others had much lower disease levels and were rated moderately resistant. These would be useful as resistance sources for breeding, without resorting to donors unadapted to South Australian conditions. Field screening could be used for resistance breeding in wheat. Without better resistance than available locally in barley, breeding for resistance using field screening would be difficult in that crop.

The fungicides flutriafol and triadimefon, which are used in South Australia to control foliar and floral diseases of cereals, were tested as seed-dressings and fertiliser-amendments for control of common root rot. Flutriafol reduced symptoms by both treatment methods, in contrast to triadimefon, which had no effect. A 25% reduction in disease incidence at anthesis and maturity resulted from flutriafol at low rates (50 parts per million of seed-dressing or 50 grams of active ingredient per hectare of fertiliser-amendment). At higher rates, about 50% reduction was observed. The fungicides were phytotoxic, causing reduction in coleoptile length, delayed emergence or reduced establishment, thickened and shortened subcrown internodes and delayed maturity. This was exacerbated by deep sowing, done deliberately to induce formation of long subcrown internodes for scoring common root rot. Mostly, yields were not affected, but were reduced in one case. Flutriafol seed-dressing reduced the frequency of isolation of *B. sorokiniana* from subcrown internodes of barley, while triadimefon did not. Applied as a fertiliser-amendment, flutriafol reduced the

population density of *B. sorokiniana* in the soil by about 55%, to about 60 ppg in barley and 40 ppg in wheat.

Population densities of *B. sorokiniana* in the soil were studied in a field experiment with rotation, soil type and tillage system as factors. There were five rotations (continuous wheat, and wheat alternated with peas, oats, grassy pasture and medic), two soil types (sandy loam and clay loam) and two tillage systems (direct drilling and conventional cultivation). Among rotations, the highest mean inoculum density was after continuous wheat, with 168 propagules per gram of soil (ppg). Peas and oats in the rotation resulted in non-significant reductions to 146 and 137 ppg respectively. Population densities after grassy pasture and medic were 108 and 104 ppg, both significantly lower than in continuous wheat. Population density was significantly lower with direct drilling than with conventional cultivation (109 versus 144 ppg). Sandy loam had a significantly higher population density than clay loam (147 versus 117 ppg). It was concluded that none of the treatments were likely to greatly reduce common root rot levels, although they could be useful in an integrated control program.

STATEMENT OF ORIGINALITY AND CONSENT TO PHOTOCOPY OR LOAN

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

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22.12.92

Date

DEDICATION

This thesis is dedicated to my wife, Virginia, who endured much during this work and supported me constantly.

Also to Dad and Pat, and many other kind people who wished me well, thanks.

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1. GENERAL INTRODUCTION

This thesis reports on a set of studies done in the state of South Australia on the wheat and barley disease common root rot. Objectives of the study were to attempt to elucidate the importance of the disease and its cause, and to indicate the potential for controlling it using resistance, rotations, tillage practices and fungicides.

Common root rot is present in most or all cereal growing areas of the world and has been extensively studied. The term "common root rot" was applied in the 1930's by Simmonds, who described it as widespread and important on the North American Prairies (Simmonds, 1941).

Affected plants occur randomly or in irregular patches in a field (Wiese, 1977). Severely diseased plants are stunted, with fewer tillers and brown to black necrosis of crown and root tissues (Sallans and Tinline, 1965), including the subcrown internode, on which severity of the disease can be assessed by the proportion of tissue affected (Ledingham *et al.*, 1973).

In various regions of North America, yield losses in wheat and barley in the 1930's were estimated to be about 5 to 10% (Machacek, 1943). Severity was greatest under conditions unfavourable to crop growth. Despite great changes over subsequent years in agronomic practices, similar losses continued to occur (Ledingham *et al.*, 1973; Piening *et al.*, 1976). Yield losses of 8 to 12% were estimated to occur in Queensland (Wildermuth, unpublished data, cited by Tinline *et al.*, 1988).

The main cause of the syndrome is *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur. The anamorph name, *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., is frequently used in the literature and is used in this thesis. *Fusarium culmorum* can cause similar symptoms and in some locations, is more frequently isolated than *B. sorokiniana* (Scardaci and Webster, 1982; Duczek, 1984). In Australia, *B. sorokiniana* is widely found (Butler, 1961), including in South Australia (Tinline,

1984; Fedel-Moen and Harris, 1987), but the role of *B. sorokiniana* in causation of common root rot in that state has not been clearly established.

Genotypes of wheat (*Triticum aestivum* L.) show no immunity to common root rot, but range in resistance from moderately resistant to very susceptible (Tinline *et al.*, 1989). Improvements in resistance have been made by breeding (Tinline *et al.*, 1989) and the presence of variability in resistance among Australian wheat varieties indicates that resistance breeding may be practicable in that country (Purss, 1970; Wildermuth, 1974).

Barley (*Hordeum vulgare* L.) is, in general, more susceptible than wheat. Genetic variability is present (Tinline and Ledingham, 1979), but ratings tend to be inconsistent (Duczek, 1984) and are discernible only at the extremes of resistance and susceptibility (Stack, 1986). Wildermuth and McNamara (1987) tested four Australian barley varieties and found no differences in susceptibility among them.

B. sorokiniana has a wide host range, infecting many members of Poaceae and some of Fabacae, Linaceae, Cruciferae, Compositae and Orchidacae (Christensen, 1922; Sprague, 1950; Sivanesan, 1987; Wildermuth and McNamara, 1987; Farr *et al.*, 1989; Wildermuth and McNamara, 1991). Sporulation is lower on some crops than on wheat or barley (Ledingham, 1961; Chinn, 1976b; Reis and Wünsche, 1984), so that rotations may reduce inoculum levels. However, their efficacy in disease control may be limited, since conidia of *B. sorokiniana* decline in viability only slowly (Chinn and Ledingham, 1958), low inoculum densities can still result in high disease levels, given conducive environmental conditions (Duczek *et al.*, 1985), and a single susceptible crop can return inoculum density to previous high levels (Wildermuth and McNamara, 1991).

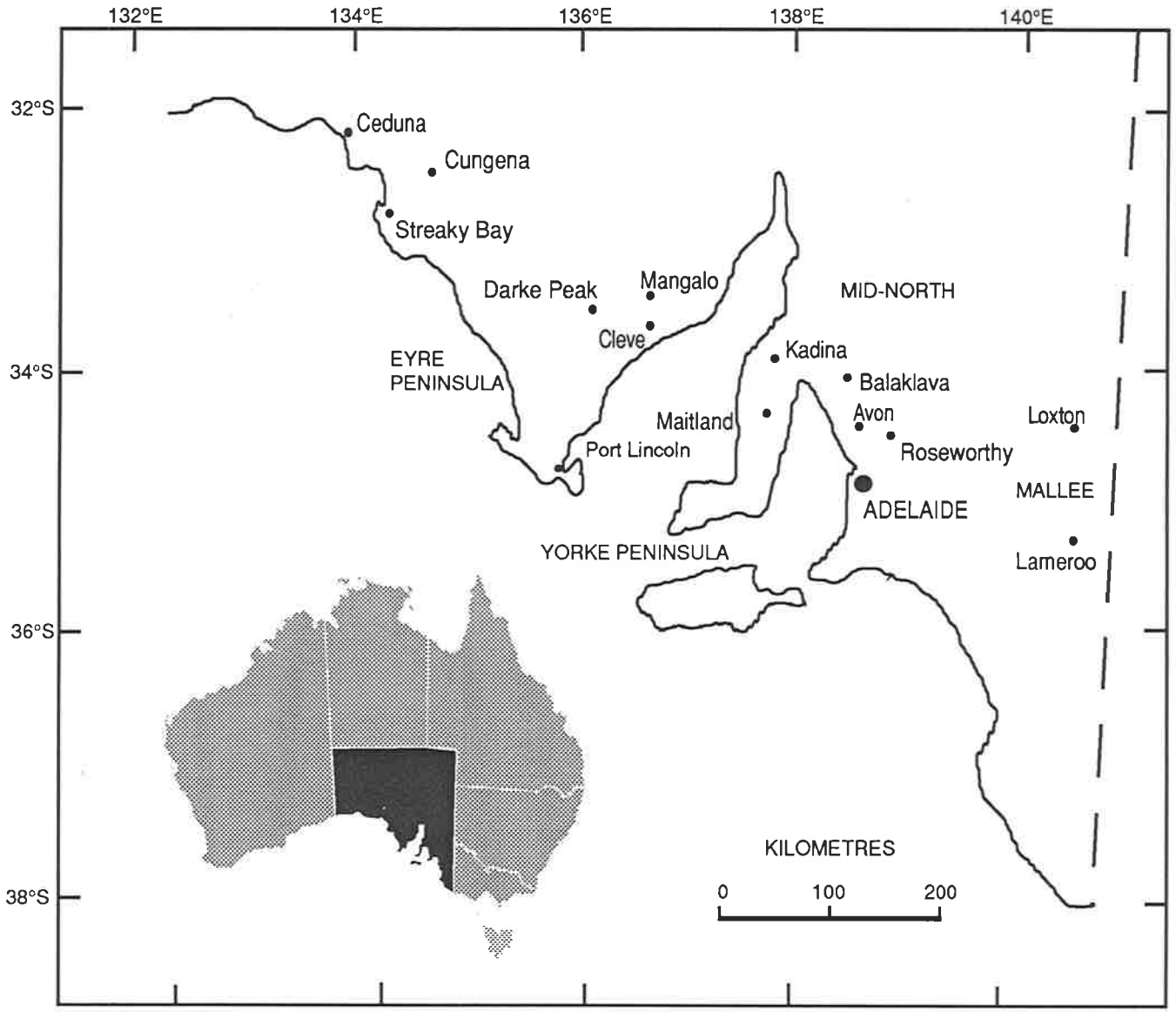
In Brazil, soil inoculum density of *B. sorokiniana* after direct drilling was about half that of conventional tillage (Reis and Abrão, 1983), but in Canada, there was no consistent effect of tillage on common root rot (Conner *et al.*, 1987).

Common root rot and *B. sorokiniana* occur on widely varying soil types through Australia (Tinline, 1984; Wildermuth, 1986), but there is little information on whether the disease varies in severity between soil types.

Systemic fungicides can reduce severity of common root rot on subcrown internodes, although without consistent effects on yield (Chinn, 1978; Verma *et al.*, 1981; Verma, 1983). Such fungicides are widely used in South Australia for control of foliar and floral diseases, and can reduce levels of take-all (*Gaeumannomyces graminis* var. *tritici*) (Ballinger and Kollmorgen, 1986a, 1986b), but their effects on common root rot there have not been reported.

Geographic features of the cropping belt of South Australia distinguish it from areas where most studies on common root rot have been conducted. In particular, the climatic conditions under which wheat and barley crops develop are quite different, so that the biology and control of the disease may be different also. The South Australian cropping belt lies approximately between latitudes 32° and 37° (Figure 1.1), which is closer to the equator than the North American Prairies and Europe, and further from the equator than the cropping regions of Brazil and Queensland. The climate of South Australia is described as Mediterranean, with hot, dry summers and cool, wet winters (Figure 1.2). Crops are sown in late autumn or early winter, grow slowly through winter using incident rainfall and mature in spring with decreasing water availability, to be harvested after about 170 days of growth in hot, dry conditions in late spring or early summer. This is in contrast to the North American Prairies, where spring wheat is planted after the thaw and grows in ever-increasing temperatures, on stored water and rainfall, to be harvested after about 100 days in fall (autumn). In Queensland, wheat and barley are grown generally through winter and spring, as in South Australia, but rainfall there is summer-dominant, early crop growth is on stored water, and humidity is higher after flowering than in South Australia. A further distinguishing factor which may affect common root rot biology is that South Australian soils are ancient, deeply weathered and leached, resulting in low fertility and trace element deficiencies.

Figure 1.1. Map of South Australia, showing some locations and centres referred to in this thesis. Other study locations are listed with map references in Appendix 1.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Balaklava
Maitland
Avon
Loxton
Port Lincoln
Roseworthy
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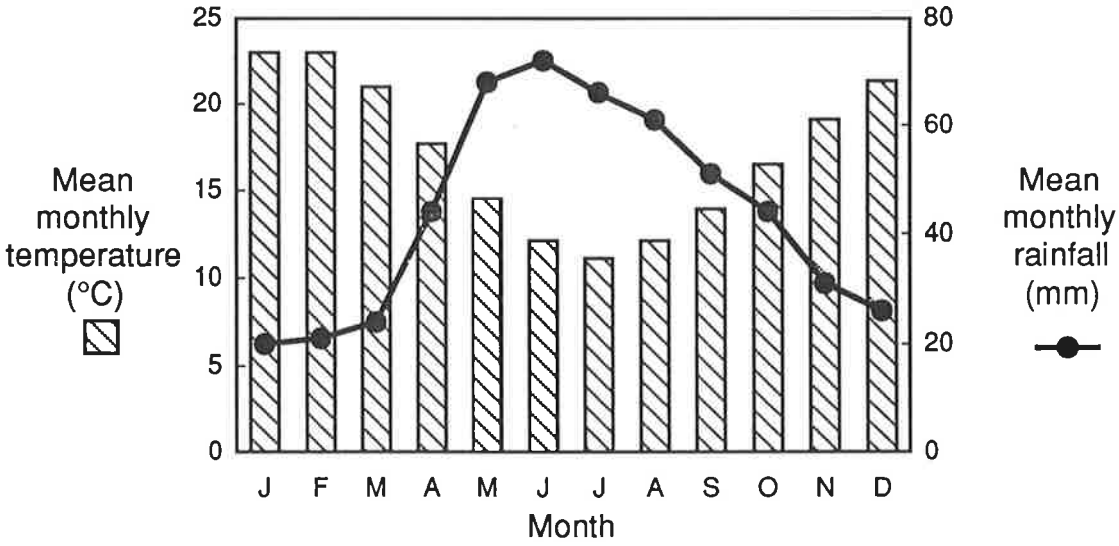
Figure 1.2. Monthly mean temperatures and rainfall in Adelaide, South Australia, indicative of rainfall and temperature patterns throughout the cropping belt.


Mean monthly temperature (°C)
25
20
15
10
5
0
80
60
40
20
0
J F M A M J J A S O N D
Month
Mean monthly rainfall (mm)

2. LITERATURE REVIEW

The wheat and barley disease "common root rot" is present in most or all cereal growing areas of the world and has been extensively studied: Harding, in a comprehensive bibliography of its causal fungus, (1980, 1981, 1983, 1986) listed almost 3,000 papers, including reviews by Simmonds (1939, 1941), Butler (1961) and Sallans (1965).

2.1. Symptoms of common root rot

The symptoms of common root rot as described by Sallans and Tinline (1965) were: brown to black necrotic lesions on the bases of the culms, leaf sheaths, crowns, subcrown internodes, seminal roots and crown roots; subcrown internodes restricted in diameter due to cortical necrosis; stunting of plants and reduction in numbers of tillers per plant. Simmonds, in the 1930's, applied the name "common root rot" to the disease, which was important on the North American prairies (Simmonds, 1941).

Simmonds *et al.* (1935) excavated root systems to examine the position and extent of infections. Lesions appeared early on the subcrown internode and primary roots (axillary and lateral) of seedlings, and crown roots were attacked as they developed. Crown and leaf symptoms appeared by midseason. Several reports have been made on progression of common root rot symptoms through the season (Verma *et al.*, 1974; Stack, 1980; Verma, 1982; Kidambi *et al.*, 1985; Bailey *et al.*, 1989). Different interpretations were reached on the nature of disease progression, but in general, disease curves were sigmoid. Bailey *et al.* (1989) related environmental factors to disease progression. Disease incidence responded to moisture when moisture was limiting, but to temperature when moisture was not limiting. Temperature was the most important determinant of rate of development of disease severity.

2.2. Scoring of symptoms

Disease severity in seedling tests can be scored on the basis of damage to roots (Cohen *et al.*, 1969; Hamilton *et al.*, 1960), but the standard procedure for scoring on mature plants is to assess the degree of necrosis on the subcrown internode. Piening (1973) referred to unpublished work showing a good correlation between the extent of lesions on the subcrown internode and on the roots of Gateway barley. Tinline et al. (1975) found that the severity of lesions on the subcrown internode was closely and negatively associated with fresh weight and grain yield. Experimental method was not reported. However, they concluded that this lesioning was a satisfactory index of common root rot.

Various scales of lesioning of the subcrown internode have been adopted (reviewed by Stack, 1992), including Horsfall-Barratt (in which categories at the extremes of the range are narrower than those in the middle) (Bailey *et al.*, 1988) and a six-category scale (Wildermuth and McNamara, 1987), but the four-category scale described by Ledingham *et al.* (1973) has become standard. The categories are *clean*, *slight*, *moderate* and *severe*, are numbered 0, 1, 2 and 3 and correspond with 0, 1 to 20, 21 to 49 and 50 to 100% of the subcrown internode discoloured. Grey and Mathre (1984) found that using the proportion of plants in the clean and severe categories was the best way to differentiate between cultivars of barley, since the slight and moderate classes were difficult to differentiate.

2.3. Causal fungus of common root rot

The cause of the syndrome was identified by Bolley (1913, cited in Simmonds *et al.*, 1950) as *Cochliobolus sativus* (Ito & Kurib.) Drechsl. ex Dastur, anamorph *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem., synonym *Helminthosporium sativum* Pamm., King and Bakke. As pseudothecia of *C. sativus* have not been reported from nature (Stack, 1992), many authors use the anamorph name, *B. sorokiniana* in preference to *C. sativus*.

Work subsequent to Bolley's, reviewed by Simmonds *et al.* (1950), elucidated the pathogenicities of *B. sorokiniana* and numerous other fungi isolated from tissue with common root rot symptoms. *B. sorokiniana* was shown to be the major causal fungus. *F. culmorum* was capable of causing a similar, but generally less severe disease. Other *Fusarium* spp. were frequently isolated, but were regarded to have lesser significance. The relative dominance of *B. sorokiniana* and *F. culmorum* among total isolates varied between areas on the Canadian Prairies (reviewed by Duczek, 1984). In Brazil, *B. sorokiniana* dominated isolates from diseased root and crown tissues of wheat and a range of Fusaria were infrequently isolated (Diehl, 1979). *F. culmorum* was also associated with root rot in California, in addition to *B. sorokiniana* (Scardaci and Webster, 1982). *F. acuminatum* and *B. sorokiniana* caused root rot in Wyoming and Colorado (Hill *et al.*, 1983), with *F. acuminatum* augmenting the effects of *B. sorokiniana*, especially after freezing temperatures (Fernandez *et al.*, 1985).

Hynes (1935) in New South Wales isolated *F. culmorum* about 60% as frequently as *B. sorokiniana* and Butler (1961) considered that *F. culmorum* could be important in the common root rot complex in Australia. However, it was an infrequent isolate in Queensland (Purss, 1970), Victoria (Chambers, 1972) and South Australia (Fedel-Moen and Harris, 1987).

The practical significance of whether *B. sorokiniana* or *F. culmorum* dominates at a given site may not be great; Duczek (1984) ranked barley lines the same for resistance at three sites which varied in relative proportions of the two fungi, although variations may occur (Tinline *et al.*, 1989).

2.4. Infection

Infection by *B. sorokiniana* can occur throughout the season (Tinline, 1977), resulting mainly from conidia (Boosalis, 1960). Histology of infections was studied by Huang and Tinline (1976). Within 24 hours of conidial germination, appressoria and infection cushions formed and penetration was made by fine infection pegs. Infection

moved from the epidermis to the cortex and endodermis, and each of these tissues were broken down. Sometimes, infection proceeded to the stele with vascular tissues becoming blocked.

2.5. Distribution of common root rot and *B. sorokiniana*

2.5.1. In countries other than Australia

B. sorokiniana has been reported from all continents and at least 58 countries (CMI Map 322, 1986).

Machacek (1943) surveyed for common root rot on wheat from 1939 to 1941 in the Prairie Provinces of Canada. No fields were free of common root rot and average disease incidence was 22, 52 and 43% in the three years respectively. While the three soil zones differed slightly each year in common root rot incidence, these differences were inconsistent between years.

A similar survey was conducted on barley on the Canadian Prairies from 1970 to 1972 (Piening *et al.*, 1976), when disease incidence ranged from 54 to 98% of subcrown internodes with lesions, with variation apparent between provinces and years. Incidence was higher than on wheat in Machacek's (1943) survey, but the contributions of species or time to this difference cannot be determined; a wheat survey was done from 1970 to 1972 (Ledingham *et al.*, 1973), but data were restricted to yield losses (see Section 2.6).

Pua *et al.* (1985) surveyed Quebec barley crops for foliar and root infection by *B. sorokiniana*. Common root rot intensity was comparable to measurements in the Prairies by Tinline and Ledingham (1979), but foliar disease was more common and severe, possibly due to the more humid atmospheric conditions in Quebec.

Common root rot was surveyed in 75 wheat crops in Brazil by Diehl (1979). Severe rotting of roots and subcrown internodes (greater than in the Canadian Prairies) was present in all crops except those where cereal crops had not been grown for more than

three years; these fields had slight to moderate infection. This study was substantiated by Diehl *et al.* (1982).

Common root rot has not been widely studied in Europe. Crown infection by *B. sorokiniana* was found infrequently in Scottish barley crops, but appeared to cause serious losses in some crops (Richardson, 1971). Jørgensen (1974) found that the frequency of seed infection by *B. sorokiniana* in Denmark was higher in years with warmer growing conditions, and it is likely that the cool growing season of central and northern Europe may limit importance of *B. sorokiniana*, compared with warmer regions such as the Canadian Prairies, Brazil and Queensland. However, where seed infection is found, it is likely that common root rot is also present and at higher levels of incidence, since Pua *et al.* (1985), in Quebec, found barley spot blotch of 8.8 and 0.5% to relate to common root rot intensity on the subcrown internode of 69.7 and 38.7% respectively.

2.5.2. In Australia

The distribution of common root rot in Australia was reviewed by Butler (1961), who stated it to be present throughout the Australian wheatbelt, at highest incidence in years when seasonal conditions were least favourable for crop growth. The majority of his references were anecdotal, rather than empirical evidence.

Samuel (1924) inspected wheat from three crops near Pinnaroo, South Australia and found high incidence of infection by *B. sorokiniana* and another *Helminthosporium* sp. (possibly *Curvularia ramosa*). These fungi were restricted to the sandhills (64% of plants yielded isolates), with no isolates from the sandy loam flats.

In a survey in New South Wales over three years, identifying the fungus from symptoms and humid chamber cultures, Hynes (1935) found *B. sorokiniana* in 28% of samples.

B. sorokiniana was found in only trace amounts in wheat at four sites in Western Australia by Chambers (1962a to e).

Wheat crops in Victoria were surveyed from 1959 to 1966 (Price, 1970) and common root rot symptoms were widespread, with 30 to 40% of roots of all plants sampled being lesioned. *B. sorokiniana* was isolated from roots less frequently than *F. culmorum* and *C.ramosa*. In most cases, the wheat followed nine years of clover ley pasture, which may be less favourable to *B. sorokiniana* than more intensive wheat rotations; Butler (1961) also considered ley pastures to have reduced the importance of the disease.

Conclusions on the occurrence of *B. sorokiniana* cannot be drawn readily from either Chambers' or Price's studies. In neither were the tissues sampled clearly specified and it is possible (probable with the latter) that roots, not crowns or subcrown internodes, were plated, thus biasing against finding *B. sorokiniana*, which is isolated less frequently from roots than subcrown internodes and crowns (Fedel-Moen and Harris, 1987). Chambers' results may be contrasted with a survey of common root rot in wheat crops around Geraldton, Western Australia in 1987 (J.M. Wilson, personal communication) in which mean incidence (percentage of plants with subcrown internode lesions) in 20 crops in one area, was 58%, while incidence from other areas ranged from 21 to 33%.

Tinline (1984) reported a survey of three regions of South Australia, in which averages of 198 propagules of *B. sorokiniana* per gram of soil and 87% incidence of common root rot symptoms were found. Disease intensity (weighted for yield loss [Ledingham *et al.*, 1973]) was 40% and isolation frequency of *B. sorokiniana* was 22%. Corroborating these findings, Fedel-Moen and Harris (1987) sampled 11 poorly growing crops of both wheat and barley in South Australia and recovered *B. sorokiniana* from 30 and 44% of subcrown internodes respectively.

A survey of wheat in Queensland showed crops to vary in incidence of *B. sorokiniana* infection from 0 to 76%, with an average of 22% (Ledingham, 1966). In 1978 to 80, another survey of Queensland wheat crops (Wildermuth, 1986) revealed the disease in all regions, varying between regions and years, with incidence ranging from 41 to

93%. Disease incidence and severity were closely correlated with soil inoculum density of *B. sorokiniana*, which was isolated from 75% of soils, at inoculum densities ranging from 0 to 320 propagules per gram. The regional variation was attributed mostly to cropping sequences (i.e. relative frequency of wheat and alternative crops), although other possible factors were soil type and period of cultivation since landclearing. The annual variation was attributed to changes in rainfall.

2.6. Effects of common root rot on yield

Common root rot was considered to be of substantial importance in New South Wales in the 1920's and 1930's, when wheat was grown for several years in succession, with individual crop losses of 25% or more occurring (Butler, 1961), although Hynes (1935) estimated the total yield loss from common root rot and take-all in New South Wales to be 4%. No data have been reported since then.

Machacek (1943) reviewed early work in North America, including field experiments in various regions, which showed grain yield losses of about 5 to 10%. In his survey of wheat crops in Manitoba from 1939 to 1941, Machacek (1943) estimated yield losses were 8.0%, 16.4% and 12.1% respectively. This equated broadly with disease incidence (Section 2.5.1). Severity seemed to be greatest when conditions for crop growth were unfavourable, due to, *inter alia*, frosted or damaged seed, deep or dense seeding, soil drifting, soil salinity, or low water-holding capacity of the soil.

Simmonds (1935) found that grain yield was reduced in moderately lesioned plants by 15 to 20%, and in severely lesioned plants by 30 to 45%, but little importance was attached to slight lesions. However, Machacek (1943) found that these caused yield reductions of 25.7%, compared to 37.5% and 57.3% for the moderate and severe classes respectively. Slightly diseased plants constituted the majority of the sample, and were responsible for the majority of the losses.

Machacek (1943) found that, in diseased plants, both number of fertile tillers per plant and weight of grain per spike were reduced. High crop density seemed to exacerbate

loss in grain yield per plant; severely diseased plants in a dense crop were always less vigorous than those in sparse crops, except, possibly, where competition was provided by vigorous weed growth.

After an interval of about 30 years, another survey was undertaken from 1969 to 71 on common root rot in wheat crops in the Prairie Provinces of Canada (Ledingham *et al.*, 1973). Since Machacek's survey reported in 1943, major changes had taken place in agronomic practices, wheat varieties and control of broad-leaved weeds by herbicides. Yield loss estimates were lower than Machacek's, although still substantial, ranging from 2.5% to 9.9% over the three years and three provinces. Yield reductions in the disease categories *slight, moderate* and *severe* were 6.0%, 12.5% and 28.2% respectively. There were corresponding reductions (both small) in number of heads per plant, as found by Machacek (1943) and thousand grain weight. Grain protein was fractionally, but probably not significantly higher in severely diseased than in healthy plants.

An increase in yield of 3.8% from common root rot was estimated in one case by Ledingham *et al.* (1973). While mean severity was low in this case, the proportion of plants in the slightly diseased category was unusually high compared to the usual distribution. Plants in this category had 12% more heads and 6% higher yield than the healthy plants. Growing conditions were very good, and Sallans (1959) proposed that this might have enabled slightly diseased plants to recover from early infection.

Piening *et al.* (1976) estimated losses in barley to have ranged from negligible to 20% on the Canadian Prairies from 1970 to 72. Losses in these years may have been lower than usual, since conditions were very favourable to crop growth, and stresses such as drought are believed to aggravate the damage from common root rot infection. There was considerable variation between years and regions. Severity seemed to have differed between the four soil types covered, although this may have been an effect of regional variety preferences, rather than soil type *per se*. Losses arose from reduced

number of heads per plant (up to 33% fewer), as found by Machacek (1943) and Ledingham *et al.* (1973).

Yield losses have also been assessed in field experiments. Piening (1973) found that among ten varieties of barley, disease incidence ranged from 61% to 96% and yield losses from 6% to 42%, due to reduced numbers of heads per plant, but the relationship between yield loss and disease severity varied between varieties. Tinline and Ledingham (1979) also found yield loss and disease severity to be inconsistently correlated among varieties of barley, but the relationship was consistent in durum and bread wheat. Barley, on average, had higher disease ratings and losses than wheat. Despite the inconsistent response in barley, Tinline and Ledingham (1979) considered that subcrown internode rating was a good measure of resistance to common root rot, since within varieties, greater disease rating on the subcrown internode consistently led to greater loss, the inconsistency being between varieties in the magnitude of their responses. In barley, later-developed yield components can compensate for a reduction in tiller number (Grey and Mathre, 1984); this effect may vary between varieties.

In the two years in common between the wheat survey of Ledingham *et al.* (1973) and the barley survey of Piening *et al.* (1976), both in the Prairie Provinces of Canada, proportional losses in barley were about 50 to 100% higher than those in wheat. Figures for the two crops varied similarly by year and province, including the case of apparent yield improvement described above. The proportional loss in each disease severity category was slightly higher (although variably so) in barley than in wheat.

Yield losses in wheat in Brazil were assessed by Diehl *et al.* (1982). Average incidence was 68% and losses were 9.1% in fields which had not been sown to wheat for at least 3 years. Higher disease incidence and yield losses (98% and 23.1% respectively) were measured where wheat was sown more frequently.

In Scotland, ears of Clermont barley were 11% smaller in plants with common root rot symptoms on the stem base than those without these symptoms, after seed was inoculated with conidia of *B. sorokiniana* (Whittle and Richardson, 1978).

2.7. Methodology for yield loss determination

Machacek (1943) argued that yield loss would be estimated more accurately by a survey of farm crops, than from experimental plots as had been the practice. He studied 60 crops in each of three years (1939 to 41), across three soil zones. Equidistant points were chosen from a map, the sites visited and the nearest wheat crop sampled. Within a crop, 10 samples, each consisting of all the plants in 1 metre of row, were taken. The plants were classified by common root rot severity, then the plants and heads in each category were counted. Bundles were threshed and grain weighed, and the mean weight of grain per plant was calculated for each disease category.

Yield loss was calculated for each field by assuming that yield of healthy plants represented potential yield without disease, by the formula:

$$\text{loss (\%)} = 100 - \left(\frac{100 \times W}{W_1 \times N} \right)$$

where N is the total number of plants, W is the yield of the sample and W_1 is the yield of healthy plants.

This approach was subsequently used routinely (Ledingham *et al.*, 1973; Piening, 1973; Piening *et al.*, 1976; Tinline and Ledingham, 1979; Diehl *et al.*, 1982; Pua *et al.*, 1985).

Machacek (1943) was concerned that the equation for yield loss estimation might over-estimate losses, since healthy plants could yield higher if adjacent to diseased plants, rather than healthy ones, which may be more competitive; thus, the assumption that healthy plants represent plant yield potential would be false. However, Tinline *et al.* (1975) tried several experimental methods to test for evidence of compensation by healthy plants, and found none.

Effects of common root rot on plant competitiveness are probably not significant until after the seedling stage (Tinline *et al.*, 1975), since incidence and severity increase

slowly at first, and then more rapidly as the plants mature (Verma *et al.*, 1974; Stack, 1980; Bailey, 1987). Verma (1973, cited in Tinline *et al.*, 1975) found that relative losses in dry weight of plants increased through the season and, in some cases, increased most rapidly just prior to maturity. This may have been related to increasing inter-plant competition. Losses in grain yield were proportionally very similar to reductions in dry weight at the final sampling.

Tinline *et al.* (1975) reviewed other aspects of Machacek's approach, including the validity of subcrown internode disease severity as a measurement of common root rot severity, the four-class severity scale and sampling for common root rot and yield near maturity, and argued all to be adequate predictions of the extent of infection and effects on plant growth.

Ledingham *et al.* (1973) advised caution in applying Machacek's (1943) formula to data pooled from several crops, since atypical results may bias results; a common root rot-free but poor crop would depress the pooled estimate of potential yield, but would not affect the pooled mean yields of diseased plants. They advised pooling mean loss estimates of individual crops, rather than the raw data. However, inaccuracy could also result from this approach if one of the crops had only a few plants in the healthy class and these were, by chance, atypically light or heavy. For this reason, estimates for severely diseased crops would be less accurate than for less diseased ones. Some form of "projection" might be advisable in these cases, such as recommended by Ledingham *et al.* (1973) and used by Diehl *et al.* (1982) for crops which had been harvested already, or which had poor seed-set due to frosting. Alternatively, these crops could be omitted from computations by some statistical process, although the crops eliminated by this process would probably have the greatest losses in grain yield.

In scoring survey samples, misdiagnosis might occur, through confusion of common root rot with take-all (*G. graminis* var. *tritici*), which is common in South Australia and also causes discolouration of subcrown internodes. Hynes (1935) studied the

comparative symptomatology of *B. sorokiniana* and *G. graminis* var. *tritici* on wheat and stated that the two were easily confused on field samples. On microscopic examination, the latter was distinguished by a freckled appearance on basal internodes and the presence of crusts of "plate mycelium". Simmonds (1941) referred to severe cases of common root rot in which plants at heading time were bleached and their heads shrivelled; laboratory diagnosis was recommended in such cases to avoid confusion with take-all. Otherwise, take-all was referred to in none of the other non-Australian studies reviewed; apparently, either co-infection of a crop by *B. sorokiniana* and *G. graminis* var. *tritici* is largely confined to southern Australia, or discrimination between the two is straightforward in other countries.

2.8. Testing the pathogenicity of *B. sorokiniana*

Hynes (1935) showed that Australian isolates of *B. sorokiniana* could cause common root rot symptoms. Elsewhere, the pathogenicity of the fungus has been well established, as reviewed by Simmonds *et al.* (1950) in the introduction to their paper. There are no complete reports on pathogenicity tests on South Australian isolates of the fungus, although Fedel-Moen and Harris (1987) claimed to have repeated in South Australia the findings of Hynes (1935). The tests by Fedel-Moen and Harris (1987) were conducted on seedlings, but generally, the disease becomes apparent late in the plant's development (Hynes, 1935). Seedling blight due to *B. sorokiniana*, which is covered elsewhere in this review (Section 2.9.1), has been studied extensively in North America, and observations during one phase of the disease cannot be extrapolated to the other. A valid test of pathogenicity under local conditions would therefore involve duplicating the field conditions which are relevant to pathogenicity and growing plants to maturity.

2.8.1. Effects of temperature

McKinney (1923) showed that soil temperature was an important determinant of the extent of infection of wheat and barley seedlings by *B. sorokiniana*. There was a gradual rise in infection with temperature, to a maximum between 24° and 32° on

both wheat and barley and little infection above that. Infection levels were low below 16° and 20° in two wheat varieties, susceptible and moderately resistant respectively, but the barley variety sustained low to moderate infection at 15°, the lowest temperature tested. Campbell (1956) and Bailey *et al.* (1987) also found that infection rose with temperature.

These results help to explain development of common root rot in South Australia. Crops are generally sown in May or June, and harvested in November or December. Temperatures in the first three to four months of growth (Table 2.1) would not favour infection or disease development, but rapid development should occur in the last two to four months of growth. Thus, temperature is an important consideration in a pathogenicity test. To simulate local conditions and to avoid seedling blight, a gradual increase in temperature should be applied (e.g. 8 weeks at 13°, then 8 weeks at 17°, then 8 weeks at 23°).

A diurnal range in temperature has little effect on disease development. McKinney (1923) found no difference in infection levels between constant 22° and a diurnal range of 14 to 30°. Burgess and Griffin (1967) found competitive saprophytic colonisation of wheat straw by *B. sorokiniana* to be much lower at 30° than 20°, but fluctuation of ± 8° around 30° reduced this difference, possibly due to temperature in part of the day, rather than none of it, being suitable for *B. sorokiniana* activity.

Table 2.1. Monthly mean minimum and maximum temperatures (°Celsius) for growing season in Adelaide (Kent Town), South Australia (9-year average to 1988, Bureau of Meteorology).

	May	Jun.	Jul.	Aug.	Sept.	Oct.	Nov.	Dec.
Min	10.1	7.6	7.2	8.3	9.3	11.3	14.0	15.5
Max	18.7	15.8	14.9	16.8	18.2	21.8	24.8	27.2

2.8.2. Effects of soil moisture

All reports on the effects of soil moisture on common root rot refer to water levels in terms of content, rather than potential energy, and so are difficult to repeat or to extrapolate between soil types. McKinney (1923) found that, in both a sandy loam and a loam, infection was favoured by high soil moisture content, although a broad range of moisture contents gave moderate to high infection ratings. Combinations of 20 to 28° and moderate soil water content resulted in high infection levels. These conditions would commonly occur in the months of September, October and November in the cereal belt of South Australia. Bailey *et al.* (1987) also indicated that moisture was an important determinant of infection at low moisture levels, although Dickson (1946) found that disease was severe in wheat and barley grown to maturity at 21.1° in both moderately dry and wet soils. This underlines the importance of measuring soil water potential in such studies; while these studies indicate that the soil-drying which often occurs in late spring in South Australia may inhibit common root rot development, it is not clear. The use of a loam or sandy loam held at a "moderate" moisture content throughout would be a valid test of pathogenicity under South Australia conditions, except for conditions near maturity. Hynes (1938) found yield losses from common root rot to be greater with low soil moisture and this relationship is generally accepted to hold true (Wiese, 1977), so the maintenance of high available water in such a test may minimise effects on grain yield.

2.8.3. Effects of growing media

Henry (1931) found that addition of trace amounts of unsterilised soil to sterilised soil reduced markedly the severity of common root rot caused by *B. sorokiniana* and reduced sporulation of the fungus in the soil. This was attributed to action of microorganisms, primarily fungi, but also bacteria and actinomycetes, in the unsterilised soil. Campbell (1956) rated common root rot at 52% in unsterilised soil and 83% in autoclaved soil. Addition to the sterile soil of several other fungi singly

with *B. sorokiniana* reduced the disease rating, although generally not as far as the level in unsterilised soil. Antibiosis and direct parasitism were demonstrated. Fungal succession has been demonstrated during which plants infected with *B. sorokiniana* were invaded by *F. culmorum* or *F. acuminatum* (Tinline *et al.*, 1975). Statler and Darlington (1972) failed to isolate *B. sorokiniana* from plants inoculated with both it and *F. culmorum.* Therefore, while the use of unsterilised soil is desirable to simulate field conditions, it might be necessary to conduct a pathogenicity test with sterile soil.

A test soil should ideally be free of, or at least low in natural inoculum of *B. sorokiniana*, in order to maximise an inoculum effect, and should be free of *F. culmorum*. However, few natural soils are free of *B. sorokiniana.* The organism is very widely distributed with a large host range including at least 79 genera of *Gramineae* (Sprague, 1950) and many dicotyledons (Sivanesan, 1987; Wildermuth and McNamara, 1987) (host range is reviewed further in Section 2.10). Conidia remain viable for several years (Chinn and Ledingham, 1958). Re-infection by airborne spores may also occur; Duczek *et al.* (1985) found airborne conidia in a glasshouse and, despite the planting medium being originally free of inoculum, 12 to 44% plants were infected with *B. sorokiniana.*

The lowest inoculum density to be expected in agricultural soils was indicated by Duczek (1981) who found spore densities of 21 to 167 viable per gram of soil in the top 10 cm of 32 summerfallow fields in Saskatchewan. Even at low inoculum densities, high levels of the disease can occur. In one test, maximum disease incidence occurred with 16 conidia per gram of soil, and incidence was high with less than 8 (Duczek *et al.*, 1985). Tinline *et al.* (1988) found little increase in disease from inoculation when the background inoculum density was 77 propagules per gram. Hence, even soil from a host-free rotation or isolated, non-agricultural soils may not have sufficiently low spore densities for use in pathogenicity tests. (The subject of natural inoculum densities is reviewed in more detail in Section 2.10).

2.8.4. Effects of inoculation method

Various workers have produced inoculum on solid substrates and mixed it into the planting medium (reviewed by Stack, 1992). Hamilton *et al.* (1960), in devising a resistance screening procedure for barley, found a sand-cornmeal inoculum caused more uniform infection than did mycelium or spores. *B. sorokiniana* sporulates abundantly on a variety of agar media and natural substrates (Stack, 1992). Spores can be scraped off, suspended in dilute salt solutions (Dodman and Reinke, 1982), sterile water (McKinney, 1923) or detergent solution (Duczek *et al.*, 1985) and mixed into the planting medium. Free conidia are the main source of field inoculum (Boosalis, 1960; Simmonds *et al.*, 1950; Sallans, 1965) so these should ideally be used in a pathogenicity test, notwithstanding the result of Hamilton *et al.* (1960). Also, a non-nutritive carrier avoids the risk of encouragement of microbial interference.

2.8.5. Effects of isolates

El-Nashaar and Stack (1989) found that pathogenicity of isolates was normally distributed, with quite small variance (all were virulent). Isolates from a long-term continuous wheat plot (Bolley's disease garden, Fargo, North Dakota) generally caused more severe symptoms than isolates from commercial fields cropped to wheat every two or three years. Hosford *et al.* (1975) showed that virulence on spring wheat was controlled by two genes, but most isolates were virulent. Races differentially pathogenic to wheat varieties have not been reported (Tinline, 1988).

Conner and Atkinson (1989) showed a degree of host specificity in *B. sorokiniana*. In pathogenicity tests on wheat and barley, wheat isolates were more virulent on wheat while barley isolates were more virulent on barley. However, the differences were minor, so that isolates from either host should be suitable in pathogenicity tests in South Australia.

To compare isolates for pathogenicity, considerable replication is required, since substantial variation in lesion severity occurs between plants. In pot tests of pathogenicity, El-Nashaar and Stack (1989) scored 30 plants in each treatment (10 for each of three replicates) and achieved acceptable variances.

2.8.6. Effects of host varieties

El-Nashaar and Stack (1989) used four varieties of durum wheat, differing in susceptibility, for pathogenicity tests of a range of isolates and found no variety-by-isolate interaction. Therefore, a single, susceptible variety of wheat could be used in pathogenicity testing.

For barley, the situation is less clear, with high variability occurring in common root rot reactions (Tinline and Ledingham, 1979; Duczek, 1984). Tinline (1988) suggested that specific isolates should be chosen for resistance screening in hosts other than wheat. However, the variability reported is within a range of susceptibility, rather than between susceptible and immune, so choice of cultivar should not affect absolute pathogenicity.

2.9. Resistance in wheat and barley to common root rot

Resistance in wheat and barley to common root rot caused by *B. sorokiniana* has been extensively studied since the 1920's and several reviews have been published, notably Hynes (1938), Simmonds (1939, 1941), Butler (1961), Sallans (1965), Tinline *et al.* (1975) and Tinline *et al.* (1989). Therefore, only reports pertinent to the present study will be discussed here.

The role of plant breeding in controlling soil-borne diseases was discussed by Tinline *et al.* (1989). Resistance was defined as the ability of a host to impede a parasite (i.e. limit its invasion, growth or reproduction) and could be rated as high, low or intermediate. They listed several factors which govern the adoption of resistance as a breeding objective. If the disease cannot be adequately and efficiently controlled by other methods; if good sources of genetic resistance are available; if the inheritance of

resistance in the host and virulence in the pathogen are known; and if reliable techniques for induction of disease, enabling accurate disease assessment, have been developed then breeding for resistance can be adopted.

2.9.1. Screening methodology

Common root rot resistance breeding would be considerably simplified if resistance could be selected for on the basis of host response to infection of seedlings, foliage or heads. Several reviews have been published covering many investigations of genetic variability in wheat and barley for resistance to common root rot (Hynes, 1938; Simmonds, 1939, 1941; Sallans, 1965; Tinline *et al.* , 1975; Tinline *et al.*, 1989). Methods used in these studies varied greatly. Inoculation methods were reviewed and further studied by Sallans (1933). Early work such as undertaken by Christensen (1922) and Ludwig *et al.* (1950) involved heavy inoculation of seed, foliage or soil under conditions conducive to infection. The resistance to intense attack which was observed, even if on seedlings or the leaves or heads of older plants, was assumed to apply to common root rot on adult plants. However, there was little success in breeding for resistance until it became apparent that seedling ratings were not indicative of field ratings on subcrown internodes of adult plants, and that ratings for the different disease forms (seedling blight, root rot, leaf spot and head blight) were not correlated and were governed by different genetic systems.

With barley, Hamilton *et al.* (1960) advocated the use of seedling tests, even though they had not tested the correlation of these with variety rankings from adult plants in the field. Clark (1966) compared resistance to three forms of barley disease caused by *B. sorokiniana*. Progeny from crosses of *Hordeum vulgare* x *H. leporinum* were tested in three ways: using the Ludwig *et al.* (1950) system, in which plants were grown in inoculated sand in warm, humid conditions in the glasshouse and rated for plant size and root length after 21 days; spraying the foliage of seedlings with conidia of *B. sorokiniana* and rating leaf infection after 5 days; and spraying conidia on heads of plants grown in the glasshouse, then plating the heads to determine levels of

infection (which was equated to head blight). All lines were susceptible to the leaf spot form of disease, although there was a wide range in susceptibility to root rot and head infection. Resistance to root rot and head infection were not correlated, indicating different genetic systems controlled the response. Cohen *et al.* (1969) studied two crosses between resistant and susceptible barleys and found no correlation between field resistance ratings on subcrown internodes of adult plants and ratings on seedlings grown in growth cabinets and inoculated by the method of Ludwig *et al.* (1950). Stack (1985) reported that three seedling disease parameters (not specified) on barley genotypes grown in the field were marginally correlated with field ratings on adult plants, but the level of correlation was insufficient to allow efficient selection in the seedling stage for common root rot resistance in adult plants. Stack (1986) also found a low correlation between the reactions of barley varieties to the spot blotch and common root rot forms of the disease in adult plants.

Similar studies have been undertaken on wheat. Harding (1971) attempted to develop a laboratory technique for screening wheat seedlings. Seeds were sprayed with dense suspensions of conidia of *B. sorokiniana* and incubated for six days before rating for damage. Genotype ratings were not correlated with previously determined field ratings and the use of seedling assessment was abandoned. Stack (1981) measured field disease ratings on seedlings and found that they were not correlated with adult plant ratings. This was supported by Bailey *et al.* (1989) who found that resistant and susceptible lines did not differ in the first 30 days in disease incidence and severity, but were readily distinguished after that. It was not clear whether disease progression was solely related to time, or whether it was influenced by plant phenology. Conner (1990) screened 18 genetically diverse wheat varieties in order to examine the relationship between resistance to common root rot and resistance to grain black point and leaf spot caused by *B. sorokiniana*. Black point was evaluated in a growth cabinet after inoculating heads with conidial suspensions in a vacuum. Spot blotch was induced by spraying plants in a growth cabinet with conidia. Root rot was tested in the greenhouse by growing plants in infested field soil for 7 weeks and rating

subcrown internode lesions by standard field test procedures. Ratings for the different forms of *B. sorokiniana* disease were not clearly related.

The same conclusion, that seedling tests give, at best, low correlations with common root rot resistance measured in the field on adult plants, seems to have been reached independently by other researchers who, without reporting their methods in detail, have screened adult plants in the field or the glasshouse for intensity of lesions on the subcrown internode (reviewed by Sallans and Tinline (1965)).

2.9.2. Variability in the pathogen and disease complex

Tinline (1988) reviewed the genetics and pathogenicity of *B. sorokiniana.* Two studies, in which pathogenicity was tested on foliage, identified genes controlling pathogenicity in wheat, barley and other gramineous species (Kline and Nelson, 1963; Hosford *et al.*, 1975). No physiological races for common root rot have been identified, but the error is comparable in order of magnitude to the effect of resistance, so races are difficult to identify. The likelihood of races occurring would be reduced by the polygenic nature of resistance (identified in wheat, see Section 2.9.3), although recombination could occur in the perfect stage (*C. sativus*), which has not been found in nature, or via parasexuality, which has been observed (Tinline, 1988). Even if a mutation or recombination to increased virulence did occur, its frequency in the population may not increase rapidly, due to the restricted dispersal of the fungus, and the ability of less virulent races to multiply substantially even on resistant varieties (moderately resistant), or on other host species (see Section 2.10). Therefore, differentially pathogenic races are unlikely to be a problem for resistance breeding.

Purss (1970) and Harding (1972) observed, in resistant accessions, that high rates of infection by *B. sorokiniana* could occur even with low disease severity. The latter author felt that this would provide selection pressure towards higher pathogenicity on resistant varieties. However, it is not clear that the fungus would gain a selective advantage (e.g. increased reproductive ability or rate) from the ability to cause higher severity. Only on genotypes which did not become infected by the wild type race,

such as some alien accessions screened by Harding (1972) and Conner *et al.* (1989), would such a mutation be beneficial to the pathogen.

El-Nashaar and Stack (1989) found that isolates from very long-term (over 90 years) continuous wheat plots caused slightly greater mean disease severity than did isolates from commercial crops in the same region. No explanation was given for this. Common varieties, not chosen for resistance and therefore probably susceptible, had been grown in the continuous plots, so there is no apparent mechanism by which selection could have occurred except differences in pathogenicity on the other species in the commercial rotations. In any case, the difference was small and probably has little practical significance.

There has been concern that *F. culmorum*, which can also cause common root rot symptoms, may complicate selection for resistance to the disease (Tinline *et al.*, 1989). However, except for an unpublished report (Tinline *et al.*, 1989), rankings of varieties were consistent in wheat (Sallans and Tinline, 1969) and in barley (Duczek, 1984) between *B. sorokiniana*-infested sites with high and low levels of *F. culmorum*. There is insufficient detail in these reports to indicate whether consistent rankings of varieties resulted from joint selection of the two resistances, or whether the same mechanism gives resistance to both fungi, or whether *F. culmorum* was simply a minor pathogenic factor in the disease complex.

2.9.3. Inheritance and sources of resistance

Studies on the inheritance of resistance to common root rot in wheat were reviewed by Tinline *et al.* (1989). Ratings ranged from moderately resistant (highly resistant or immune wheat varieties have not been identified) to very susceptible and were repeatable among sites and years. Several studies (Sallans and Tinline, 1965; McKenzie and Atkinson, 1968; Larson and Atkinson, 1981; Stack, 1982; Bailey *et al.*, 1988) indicated polygenic resistance, although the number of genes seemed to be small. Genes varying in dominance were found and located on chromosomes 2B, 2D, 5B and 6A. Bailey *et al.* (1988) measured heritability of resistance in two crosses and

concluded that selection could be conducted in the F_3 in some cases, but in general would be more effective from the F_4 or F_5, when the higher proportion of homozygous genotypes results in greater genetic variation in the population. Tinline and Harding (1985) intercrossed a set of highly resistant lines and selected single plants by progeny testing in the F_3 to F_6 generations. The proportion of selections that were more resistant than their parents increased from 21% in the F_3 to 68% in the F_6. DePauw *et al.* (1984) registered nine spring wheat germplasm lines, selected for resistance and agronomic traits from crosses between highly resistant lines.

Harding (1974) screened 7,500 wheat accessions and found that no geographical region was preeminent as a source of high resistance, although Canadian varieties were generally more resistant than those from other regions, presumably due to selection against extreme susceptibility. Purss (1970) found that a small set of Australian varieties were generally intermediate in their reactions, compared with Canadian and other lines of known susceptibility and resistance. Wildermuth (1974) confirmed these results, but also found one variety, Festival, to be among the most resistant, so he suggested that breeding for resistance was an option for minimising the effect of the disease in Queensland. A range of resistance was found among Australian cultivars (Wildermuth and McNamara, 1987), some of the more resistant having common parentage.

Alien species have received attention as potential sources of resistance for wheat. Harding (1972) screened 40 accessions from 14 species of *Triticum* and found resistance in all three ploidy groups. Resistance was measured by both disease severity and infection frequency by *B. sorokiniana*. All of the diploids and tetraploids developed relatively low levels of disease (i.e. they were moderately resistant), but the hexaploids ranged from moderately resistant to very susceptible. However, the diploids differed from the other ploidy groups, in having much lower levels of infection. Similarly, Agrotana, an amphiploid of wheat and *Agropyron trichophorum* (a diploid), had very much lower infection rates by *B. sorokiniana* than the moderately resistant bread wheat Neepawa, although they had comparable disease severity (Conner *et al.*, 1989). Wildermuth (1974) found an Emmer to be more resistant than a set of bread and durum wheats. Stack and McMullen (1979) found

that most durum wheats were more susceptible than the resistant bread wheat, Thatcher, but two varieties were considerably more resistant.

Screening for resistance to common root rot in barley has been much less encouraging. The range of susceptibility seems to be narrower than in wheat and the results are less consistent. Early studies were reviewed by Cohen *et al.* (1969), but none of these were applicable to breeding for resistance to common root rot, due to their reliance on the expression of seedling and foliar disease. The only applicable genetic analysis reported was by Cohen *et al.* (1969). In one cross tested in the field, in which the parents were resistant and susceptible, the F_3 distribution extended beyond the range of the parents, but this apparent genetic segregation could not be analysed further due to the form of the experimental design. In another cross, in which both parents were susceptible, the distribution of the F_3 exceeded that of the parents in both directions, but the authors considered that this was an artefact of other traits such as time of maturity and tillering capacity, rather than an expression of inherent differences in susceptibility. Piening *et al.* (1969) demonstrated less root rot on two varieties than on two others. This was confirmed by Tinline and Ledingham (1979) with a larger set of varieties, although ratings were generally higher than in wheat. Duczek (1984) compared 6-row and 2-row accessions in several tests and found that root rot ratings were variable, but that rankings were consistent. Resistance ratings were narrower in range and less consistent between tests in the 2-row than in the 6-row accessions.

Grey and Mathre (1984) tested 16 varieties, some 6-row and others 2-row, in two field sites. Among the 2-rows, the range of susceptibility was narrow, but some varieties were slightly more resistant than others. Consistent family reactions provided evidence that resistance was a heritable trait. Stack (1986) found that only at the extremes of resistance and susceptibility of 6-row barleys were rankings consistent between separate field tests. Four barley varieties tested by Wildermuth and McNamara (1987) developed severe common root rot.

2.9.4. Relationship of resistance and susceptibility to yield loss

Tinline *et al.* (1975) reported that unpublished data showed direct relationships between the severity of subcrown internode lesioning and reductions in fresh weight and grain yields. This was supported by Ledingham *et al.* (1973) and Verma *et al.* (1976) for wheat, and Piening (1973) and Piening *et al.* (1976) for barley. Such results support adoption of common root rot resistance, measured by a subcrown internode index, as a breeding objective. Tinline and Ledingham (1979) conducted field tests on several varieties of wheat and barley at several sites over several years, to determine whether this relationship was consistent between varieties. Individual plants were scored for disease and their yield was measured. Losses were calculated by comparing yields of diseased plants with those of healthy plants. In both wheat and barley, mean disease ratings and yield losses varied considerably between sites and years. In wheat, even though losses did not differ significantly between varieties in most tests, disease severity and yield loss were significantly correlated in all 11 tests. The relative performance of varieties was quite consistent, with the exception of some apparently spurious results. The relationship in barley was less close, with a significant correlation occurring in three of six tests. Compared to a standard variety (Bonanza), some varieties (Conquest, Betzes and Galt) seemed to express tolerance, having high disease severity but low losses. Piening (1973) made a similar observation in one field test. Other lines were variable in their losses, relative to the standard, possibly indicating that environment substantially conditioned response to the pathogen. These results indicate that improvement of variety yields through selection for resistance using a subcrown internode index should be straightforward in wheat, but not in barley.

2.10. Effects of rotations, tillage and soil type on common root rot

In a series of controlled environment pathogenicity tests conducted by Duczek *et al.* (1985), incidence and severity of common root rot generally increased in proportion to inoculum density of *B. sorokiniana*, but did not reach their potential maxima (i.e.

100%). The inoculum density at which disease parameters reached their maxima varied between tests, apparently depending on environmental conditions. Tinline *et al.* (1988) deduced this upper threshold to have been between 77 and 130 propagules per gram of dry soil in field experiments, which was the natural density at which adding inoculum did not increase common root rot levels. Inoculum densities in South Australia are often higher than this (Tinline, 1984; Whittle, unpublished data). For a treatment to be effective in reducing common root rot, it must reduce the soil inoculum density of *B. sorokiniana* below the level at which maximum disease occurs. This may not occur if initial populations are high, or if a high proportion of conidia survive the treatment. The involvement of environmental factors in the relationship (Duczek *et al.*, 1985), means that the effect of a treatment in the field may be inconsistent between years and locations.

2.10.1. Longevity of conidia

Chinn and Ledingham (1958) measured conidial longevity by collecting infected stubble at a range of times after conidia were assumed to have formed. Conidia were washed off and cultured. Mean germination was 90% after seven months, but fell to 40% after 12 to 22 months. Chinn (1965) showed soil inoculum density to decline during a summerfallow in Saskatchewan, from 70 conidia per gram to 52 before winter, and 36 after it. Duczek (1981) found that viability of conidia decreased down the soil profile, possibly partly due to conidial mean age increasing with depth.

Conidial longevity is enhanced by fungistasis (Chinn, 1953), with low germinability overcome by addition of plant tissues (Christensen, 1926) or a range of nutritious substances (Chinn and Ledingham, 1957).

Survival depends on several environmental factors. Chinn and Ledingham (1958) placed conidia in a soil (unspecified) which was then held at a range of water-holding capacities (soil water characteristic not given) and showed that the period of viability was reduced by increased moisture content. This was supported by Ledingham (1970). Garrett (1975) found that the median saprophytic period in soil at 20° varied

between isolates and between batches of straw used in colonization experiments, and that addition of $NaNO_3$ reduced viability of the fungus in straw buried in soil.

Environmental effects on conidial viability might be indirect, through influencing antagonistic microflora/fauna. Garrett's finding on $NaNO_3$ was attributed to microbial competition. Domsch *et al.* (1980) reviewed this, giving examples of antagonism or parasitism by bacteria, actinomycetes, other fungi and a giant soil amoeba. *B. sorokiniana* was parasitised by an amoeba (Duczek and White, 1986) and a fungus (Duczek, 1986).

2.10.2. Effects of rotations

Whether manipulating crop rotations can control common root rot depends partly on the rate of inoculum decline in the absence of a host (see Section 2.10.1), and on the abilities of *B. sorokiniana* to first infect and then reproduce on the component crops of the rotation.

B. sorokiniana can infect a wide range of plant species, including many members of Poaceae and some of Fabacae, Linaceae, Cruciferae, Compositae and Orchidacae (Christensen, 1922; Sprague, 1950; Sivanesan, 1987; Wildermuth and McNamara, 1987; Farr *et al.*, 1989).

Fewer species and varieties have been studied for their effects on fungal reproduction and inoculum potential. Barley is a very good host (Chinn, 1976b; Kidambi *et al.*, 1985; Piening and Orr, 1988), so that barley crops tended to have more severe common root rot in a barley-barley rotation compared to other rotations (Piening and Orr, 1988). Inoculum production on barley is higher than that on wheat (Chinn, 1976b), although wheat allows high sporulation relative to other crops (Ledingham, 1961; Chinn, 1976b; Reis and Wünsche, 1984). Wheat varieties vary in their support of sporulation (Chinn, 1976b).

There are several reports on the effect of oats on inoculum levels. Sporulation on oat residues was lower than on wheat, but substantially higher than on broadleaved crops

(Reis and Wünsche, 1984). Soil inoculum densities after oats were lower than after wheat (Ledingham, 1961; Chinn, 1976b; and Piening and Orr, 1988; Reis and Wünsche, 1984). Despite the inoculum reduction, Chinn (1976b) questioned the value of oats as a cleaning crop, since sufficient inoculum remained to cause high disease levels. However, this reduction in inoculum potential led to a reduction in disease parameters to a slight degree on wheat (Ledingham, 1961) and substantially on barley (Piening and Orr, 1988). Therefore, oats might contribute to common root rot control, in rotation with other poor hosts and moderately resistant wheat and barley varieties.

Butler (1961) considered oats to be resistant to *B. sorokiniana*, but favourable to the multiplication of *F. culmorum*, which is also a common root rot pathogen (Duczek, 1984) (see Section 2.3). Butler's contention was supported by Sturz and Bernier (1987), who were unable to isolate *B. sorokiniana* from oat crops in a survey, despite it being common on barley. They found oat isolates were dominated by *F. culmorum*. Poor control by oats of common root rot was ascribed by Butler (1961) to this effect on *F. culmorum*, but this was based on conjecture. There has been no survey of the occurrence of this fungus in southern Australia although it now appears to be uncommon (Chambers, 1972; Fedel-Moen and Harris, 1987).

Reports on rye are conflicting. Some have found it to be less susceptible than wheat and barley (Christensen, 1922; Diehl, 1983; Chinn, 1976b), but Wildermuth and McNamara (1987) showed high infection rates. It can support abundant sporulation of *B. sorokiniana* (Reis and Wünsche, 1984), leading to high soil inoculum density (Reis and Wünsche, 1984; Chinn, 1976b). It therefore has no role as a break crop for common root rot control.

Reactions of 112 grass species to foliar inoculation with *B. sorokiniana* ranged from immune to very susceptible (Christensen, 1922), but there are no reports which indicate grasses to have exacerbated common root rot levels when in rotation with susceptible cereal crops. Grass species tested by Ledingham and Chinn (1964) and

Wildermuth and McNamara (1987) were less susceptible to root infection than wheat. Low sporulation occurred on residues of ryegrass (*Lolium multiflorum*) (Reis and Wünsche, 1984) and low common root rot levels in wheat crops followed bromegrass (*Bromus unioloides*) (Piening and Orr, 1988).

Non-poaceous crops could generally be considered as break crops for common root rot, with the reservation that conidia have a high persistence rate (see Section 2.10.1). Reis and Wünsche (1984) found considerably less sporulation, and in some cases none, on residues of 10 broadleaved species than on wheat, although sporulation on alfalfa/lucerne (*Trifolium sativum*) was intermediate. Rape/canola was susceptible to infection by *B. sorokiniana* in a glasshouse test (Wildermuth and McNamara, 1987), but Sturz and Bernier (1987) were unable to isolate the fungus from rape crops, despite it being endemic in the survey area. Sporulation did not occur on rape residues (Reis and Wünsche, 1984). Soil inoculum density after rape was low compared to that after wheat (Chinn, 1976a; Reis and Wünsche, 1984). Disease levels were reduced slightly in subsequent wheat crops (Piening and Orr, 1988), although there was no reduction in a bioassay with wheat seedlings in a glasshouse (Chinn, 1976a).

2.10.3. Effects of tillage

Soil inoculum density of *B. sorokiniana* with direct drilling was about half of that following conventional seeding (Reis and Abrão, 1983). This effect seems to be partly related to intensity of tillage, since even with "minimum tillage", common root rot ratings were lower in only two of 11 years, compared to conventionally cultivated plots (Conner *et al.*, 1987).

The lower inoculum density with direct drilling might be due to several factors. One is reduced mechanical dispersal of inoculum; vertical dispersal was lower in direct drilling than in conventional cultivation treatments (Duczek, 1981; Reis and Abrão, 1983). Also, inoculum production may be restricted to near the soil surface, due to the shallow sowing which is inherent in direct drilling systems.

Shallow sowing might lead to less infection (Broadfoot, 1934), since less tissue is exposed to the fungus. Increases in common root rot with depth of seeding have been shown in spring wheat by Greaney (1946) and Tinline (1986) (depth range 2.0 to 7.5 cm), in winter wheat by Broscious and Frank (1986) and in barley by Duczek and Piening (1982) (depths 3.0 and 6.0 cm) and Piening *et al.* (1969). Broadfoot (1934) did not find this association in spring wheat, perhaps because only a limited range of depths were assessed.

Direct drilling has many aspects which might influence sporulation and persistence of conidia. These include earlier sowing dates, changes in water relations, increased soil organic matter content, improved soil structure, either increased or reduced bulk density, different soil biota, greater herbicide use and reduced aeration. However, these aspects of direct drilling and tillage have received little attention in the context of rate of infection by *B. sorokiniana.* Reis and Abrão (1983) postulated that lower inoculum in direct drilling than in conventional cultivation was due partly to higher moisture in the latter, allowing increased sporulation, and partly to higher antibiosis in the zero tillage treatment, but no evidence for either hypothesis was given. The range of water potential favouring sporulation has not been reported, so this hypothesis is unsupported. Rao (1959) and Burgess and Griffin (1967) found *B. sorokiniana* could be a moderate competitive saprophyte, contrary to the finding of Butler (1953). Burgess and Griffin suggested that Butler's finding was the result of the use of a soil with high organic matter content, this organic matter increasing the activity of the soil microflora. In this respect, Reis and Abrão may have been correct.

2.10.4. Effects of soil type

Common root rot and *B. sorokiniana* occur on widely varying soil types common through Australia (Tinline, 1984; Wildermuth, 1986), although near Pinnaroo in South Australia, Samuel (1924) found high incidence of infection of wheat by *B. sorokiniana* on sandhills, but none on the sandy loam flats. Duczek (1981) reported that in Saskatchewan, inoculum density did not differ between soil colours and

textures in a survey, but that percentage germination of conidia was lower in one colour than in the others.

2.11. Effects of fungicides on common root rot

Non-systemic fungicides applied as seed dressings can reduce seedling blight caused by *B. sorokiniana* (Hampton, 1978), but systemic activity may be required to reduce common root rot (Verma *et al.*, 1981). Two systemic fungicides, imazalil and triadimenol, have been reported as suppressing the disease (Chinn, 1978; Verma *et al.*, 1981; Verma, 1983; Piening *et al.*, 1983; Verma, 1986). In preliminary studies, there was less sporulation from tissues of treated plants than untreated ones (Verma *et al.*, 1981).

Characteristics of triadimenol and other triazole fungicides were reviewed by Scheinpflug and Duben (1988) and Carlile (1988). These fungicides belong to the demethylation inhibiting (DMI) group of sterol-biosynthesis inhibitors, which are active against a broad range of fungi at low application rates. Triadimenol has a high level of activity against soil-borne *B. sorokiniana*. After application to the seed, it penetrates the pericarp when the seed becomes wet after sowing and mostly enters the embryo. Translocation is acropetal, so very little enters the roots systemically, although the roots may absorb some of the fungicide which has moved from the seed into the soil. Buchenauer (1990) described the modes of action in detail, but further discussion of this is not required here.

Despite control of common root rot, at least on the subcrown internode and crown, if not on the roots, by these fungicides, there was no consistent yield improvement; conversely, phytotoxicity was often observed (Chinn, 1978; Verma, 1983; Verma *et al.*, 1986). One manifestation of this was an increase in diameter and reduction in length of the subcrown internode (Chinn, 1978; Verma *et al.*, 1986). These growth regulation effects, which are common with DMI fungicides, can be exacerbated by deep sowing in heavy, wet, cold soil, resulting in delayed emergence (Scheinpflug and Duben, 1988), and common root rot experiments are often sown deep

deliberately, to induce formation of a long subcrown internode for disease scoring. If the crop is not sown at excessive depth, these growth regulation effects may be beneficial, since shortening of the subcrown internode gives better lodging resistance, longer and more branched roots, more and stronger tillers, and better resistance to high temperatures and drought. McMullen and Stack (1987) found that wheat and durum seedling stands were denser and more vigorous with such treatments.

Losses of more than 20% of active ingredient can occur with dry seed treatment through poor adherence, and better activity is achieved with wet formulations (Scheinpflug and Duben, 1988).

Because significant fungicide concentration does not occur in the roots of plants treated by seed-dressing, applying fungicide to the root zone, rather than on the seed, might improve protection of the roots. It may also reduce the phytotoxic effects, by allowing slower uptake of the fungicide into the plant. Chambers (1964) reported that fungicides mixed with fertilisers and applied in-furrow at seeding time were unsuccessful in controlling take-all. Application of triazole and other fungicides to soil by drenching or incorporation or tillage for control of take-all was reported by Bateman (1980, 1981, 1984 and 1985) and Bateman and Nicholls (1982). Ballinger and Kollmorgen applied fungicides including triazoles to the soil, using, as carriers, clay granules and pellets of denatured wheat seeds (1986a, 1986b) and superphosphate granules (1988). Take-all severity was reduced and wheat yields improved. Similar work on blackleg (*Leptosphaeria maculans*) of rapeseed (*Brassica napus* and *B. campestris*) was reported by Ballinger *et al.* (1988a, 1988b) with another triazole, flutriafol. Goos *et al.* (1989) compared the action on common root rot of imazalil impregnated into monoammonium phosphate and applied as a seed dressing. Suppression of disease by imazalil dressed on seed was lost with the fertiliser-impregnation method.

3. GENERAL METHODS

The following methods were used routinely, except where specified.

3.1. Field trials

Field trials were generously conducted by the wheat breeding programs of the Waite Agricultural Research Institute (Dr. A.J. Rathjen) or Roseworthy Agricultural College (Mr. G. Hollamby), or the regional agronomy system of the South Australian Department of Agriculture. The descriptions which follow apply to each, their major differences being in plot dimensions and site layout. In general, practices were as close as practicable to typical, local agricultural systems (Taylor, 1991). Trial sites on farms were chosen accordingly, and were marked out after preliminary ground preparation by the farmer. From that point, management (sowing, fertilisers, herbicides and harvesting) was done by the wheat breeding group.

Trials in the wheat breeding programs (noted in text) were sown with a combine seeder modified with a magazine system and cone-seeding devices to sow three adjacent plots at a time, interspersed with single non-sowing rows. Plots were 60 cm wide with 4 rows spaced by 20 cm, and 5.2 m in length. In the Agronomy Group trials (noted in text), plots were sown singly, 10 m long by 8 rows wide, spaced by 20 cm. Seed was placed at between 4 and 8 cm depth, to induce the formation of long subcrown internodes, on which common root rot was scored. The standard sowing rate was 100 kg/ha, using seed which was from either certified pure crops or field trials of the previous year, and graded to at least 2.5 mm diameter. Fertilizer was generally applied into the furrow at 100 kg/ha, as Topfos® double superphosphate (16.2% available phosphate). Experiments were bordered on their sides by buffer plots of the same species. Herbicides were applied as required. Typical usage was:

* ryegrass (*Lolium* spp.)- 1.0 l/ha of Hoegrass® (diclofop-methyl, 375 g a.i./l)
* wild oats (*Avena fatua*) - 1.5 l/ha of Hoegrass®

* broad-leafed weeds - 1.0 - 1.4 l/ha Buctril M® (bromoxynil, 200 g a.i./l) or 7.0 g/ha Ally® (metsulfuron-methyl, 600 g a.i./kg) or 6.0 g/ha Ally® + 100.0 ml/ha MCPA (4-chloro-2-methylphenoxyacetic acid).

Plots were harvested with a specialised header, and grain removed in paper bags for later weighing.

3.2. Fungal isolations

3.2.1. Non-selective isolation from tissue

Roots and crowns were washed under a water jet until free of soil. Representative specimens were cut into pieces 2 to 5 mm in length. Half were surface-sterilised prior to plating, by shaking for 1 minute in Milton's solution (1% available chlorine as sodium hypochlorite), then rinsing twice by shaking in sterile distilled water. The remainder were not surface-sterilised. Specimens were plated on 1/6-strength nutrient Dox yeast agar (NDY/6) (Warcup, 1955) supplemented with 100 µg/ml streptomycin sulphate and 20 µg/ml chlortetracycline hydrochloride. Plates were incubated at 15°C for 2 to 3 days. Transfers to NDY/6 were made if necessary to purify and identify isolates.

3.2.2. Selective isolation of *B. sorokiniana* from tissue

In all field trials prior to scoring, representative samples of lesioned subcrown internodes were examined for the presence of *B. sorokiniana*. Subcrown internodes and crowns were first washed under a water jet until free of soil. Specimens were then surface-sterilised as above, and were either placed in humid chambers and left by the window to allow conidia to form, or plated on selective medium. The medium used was that of Dodman and Reinke (1982), modified to include 70 ppm rather than 700 ppm of rose Bengal, to reduce inhibition of *B. sorokiniana* (the medium is referred to as DR70 in this thesis). Early in the study, specimens were plated directly on the medium. Later, to achieve greater selectivity, they were plunged into the medium, or covered with it using an agar sandwich (Chapter 5).

3.2.3. Selective isolation of *B. sorokiniana* from soil

Soilborne inoculum of *B. sorokiniana* was quantified by soil plating on DR70 medium (Dodman and Reinke, 1982). One hundred grams of soil was dried at 35° overnight. A subsample of 10.0 g was then placed in a 250 ml conical flask with 100 ml of 0.1% NaCl solution. The flask was shaken vigorously on a mechanical shaker for 20 minutes. An aliquot (0.5 ml) was drawn by pipette immediately after shaking and expelled onto solidified DR70 medium in 90 mm diameter, round Petri dishes, then spread with a sterile glass rod over the entire agar surface. Plates were incubated in light or dark at 20° for 7 to 8 days.

3.3. Sampling and scoring of common root rot

Samples for scoring common root rot were taken in some experiments on two occasions, anthesis (Zadoks scale 61 to 69) and mid-dough (Zadoks scale 85) (Zadoks *et al.*, 1974), and in other experiments, at the latter stage only.

Plants were sampled randomly through plots, by grasping and gently uprooting the plants, where necessary loosening the soil with a spade. From 1984 to 1987, plants were taken in predetermined, arbitrary numbers, usually 20 per plot. After that, pilot studies were done on buffer plots; where fewer than 50% of plants in a sample of about 200 had lesions, 35 plants per plot were taken, otherwise, 20 plants.

Disease was scored on the scale of Ledingham *et al.* (1973) for subcrown internode discolouration (*clean*, *slight*, *moderate*, and *severe*, or 0, 1, 2 and 3, which correspond to 0%, >0 to 20%, >20 to 50%, >50 to 100% of tissue discoloured) (Figure 3.1). Anthesis samples were scored shortly after sampling, but mid-dough samples were scored through January, February and March, between harvest and the subsequent cropping season. Plants were kept in a dry room prior to scoring, and were turned until dry to prevent rotting after sampling.

3.4. Statistical methods

Common root rot data were expressed as incidence (percentage of plants with lesions) and severity. Percentage data were submitted to arcsine transformation ($x = \frac{180}{\pi} \arcsin \sqrt{x\%}$) prior to analysis, and means were detransformed for presentation.

Analysis of variance (ANOVA) was performed using the GENSTAT 5© statistical package (Lawes Agricultural Trust, Rothamstead Experimental Station) on a DEC VAX/VMS computer. Plots of residuals versus fitted values were made and if warranted, data were transformed to remove heteroscedasticity. A range of other methods were used as required, and these are described in the relevant chapters.

3.5. Other

Plant growth stages were scored using Zadoks scale (Zadoks *et al.*, 1974).

Place names mentioned in the text are listed with map references in Appendix 1, and can be located on Figure 1.2.

Figure 3.1. Four category scale used for scoring lesions on subcrown internodes (Ledingham *et al.* 1973). Categories from left are *clean* (0), *slight* (1), *moderate* (2) and *severe* (3). Photograph reproduced by kind permission of Agriculture Canada.

Figure 1. Symptoms of common root rot on subcrown internodes of mature wheat plants; left to right, severity classes clean, slight, moderate, and severe.

4. DISTRIBUTION OF COMMON ROOT ROT IN WHEAT AND BARLEY CROPS

4.1. Introduction

The first published report of common root rot in South Australia was by Samuel (1924), who studied its distribution in wheat crops near Pinnaroo and found high incidence of infection by *B. sorokiniana*. There were no further studies of its potential importance until Tinline (1984) reported a survey of three regions of the state, showing very high incidence of symptoms, and high densities of *B. sorokiniana* in the soil, comparable to those which cause severe disease in Canada (see Section 2.5). Fedel-Moen and Harris (1987) reported frequent isolation of *B. sorokiniana* from subcrown internodes of wheat and barley crops.

Besides the studies of Samuel (1924), Tinline (1984) and Fedel-Moen and Harris (1987), common root rot may have been systematically overlooked in other investigations in southern Australia, since the methods used with the more visually obvious diseases, cereal cyst nematode (*H. avenae*), take-all (*G. graminis* var. *tritici*) and Rhizoctonia barepatch (*Rhizoctonia solani*), may not be appropriate for common root rot. For example, the studies of McKinney (1923) indicated that little disease development would occur at temperatures below 15°, and these temperatures prevail in the winter months (see Section 2.8.1), when many studies are undertaken. In mature crops, the disease may be mistaken for "haydie" caused by the take-all fungus (*G. graminis* var. *tritici*), because both common root rot and take-all can cause brown to black discolouration of the subcrown internode, and incorrect diagnoses may be made where fungi are not isolated (Price, 1970; Butler, 1961)

This chapter reports on surveys undertaken to provide more extensive information on the nature and distribution of common root rot in South Australia, and the factors, such as climate, soil type and farming practices, which influence importance of the disease. Also, progression of common root rot through the season was studied, to indicate better when investigations of the disease should be made.

4.2. Materials and Methods

Two types of surveys of common root rot were conducted in South Australia in 1988 and 1989. One was a qualitative survey of barley crops, with the objective of mapping the distribution of the disease across the entire cereal-growing area of the state. The other was intended to quantify incidence and severity of common root rot in wheat and barley crops. A study of development of common root rot through the growing season in wheat at six field sites was made, and as the results supplement the findings from the surveys, they are presented here.

4.2.1. Qualitative survey for mapping common root rot

The mapping survey was done using a system developed by Hirsch and Manton (1989). Mapping units were geographical subdivisions called Hundreds (100 square miles), for which accurate production data are collected by the Australian Barley Board. Two teams of two surveyors, one for Eyre Peninsula, and one for the area east of that, took random samples of 3 to 5 plants at about 20 points on circular transects through crops in September and October of 1988 and 1989. The samples were scored on site for presence or absence of common root rot symptoms in the total sample. There were 1,168 crops surveyed in 1988 and 915 in 1989. The area of each crop was estimated and data were collected on portable computers. The proportion of each Hundred's barley area in which symptoms were found was calculated. These data were transferred to the Atlas Graphics System® computer application and mapped.

4.2.2. Quantitative survey of disease levels and yield losses

The survey for quantification of common root rot in wheat and barley crops was conducted in seven geographical regions of SA in October and November, when crops were expected to be in head and ripening, in 1988 and 1989. Samples were taken from 49 crops of barley and 128 of wheat in 1988, and in 1989, from 65 crops of barley and 100 of wheat. In order to take samples at similar growth stages, the survey commenced in early maturing areas and finished where crops are harvested

later. However, even within regions, crops varied considerably in growth stage, and some had already been harvested. Plants were sampled regardless of growth stage.

4.2.2.1. Selection of survey sites

Survey areas were selected to represent the farming regions in SA, in terms of gross climatic and edaphic characteristics. Seven regions were demarcated for general consistency of climate and soil type, although there was heterogeneity on a local scale (Table 4.1). Prior to sampling a region, a route was planned to cover the area uniformly. Variations to the route were occasionally made when arability/intensity of land use was low and crops few. In areas of intense cropping, every third crop was sampled. When crops of either species were infrequent, a higher proportion was sampled. The number of crops sampled in each region is listed in Table 4.1.

4.2.2.2. Sampling technique

Crops were entered 50m from a fence corner to avoid headlands and unevenly sown areas. A transect was commenced 20 paces into the crop, consisting of a circle of 50 to 100m diameter. Each 10 paces approximately, a handful of plants (2 to 10) was uprooted. Samples were taken at random except where subcrown internodes were too short to be scored (due to shallow sowing) or take-all patches were encountered (since this disease can cause lesions on the subcrown internode and therefore confuse scoring of common root rot). The first sample was examined and if subcrown internodes were short or non-existent, this sample was discarded and the rest were taken from deeper sown rows growing from seeding ridges. In 1988, total sample sizes of about 150 plants per crop were taken, approximately the same as taken by Tinline and Ledingham (1979), but this was increased to about 200 to 250 in 1989. Crop samples were bundled, labelled with their location and taken to the laboratory for measurement. Most samples were mature when taken, but where necessary, samples were allowed to air dry before processing.

4.2.2.3. Assessment of common root rot

Plants were scored for severity of common root rot (Chapter 3). Plants with no subcrown internode, and those which had black stelar lesions in the seminal roots, indicative of take-all, were discarded. Crop mean values of incidence (percentage of plants with lesions) and severity were calculated.

In the first year, the variety of each wheat crop was determined, using head and grain characteristics (MacIndoe and Walkenden-Brown, 1968; Ferns *et al.*, 1975; Fitzsimmons *et al.*, 1983) and electrophoresis banding patterns of seed glutenins (Lawrence, 1986). Varieties were not characterised in the second year, since disease levels were low and varietal patterns of disease incidence were unlikely to have been distinguished.

Samples were grouped into geographic regions. Incidence and severity data were analysed, with year (1988 vs. 1989), crop (wheat vs. barley) and region as factors. Three regression analyses, fitting the factors in different orders, were performed with GENSTAT 5© to give accumulated ANOVA's. This procedure was required (L. Giles, personal communication) since the data matrix was non-orthogonal, due to uneven sample numbers in the regions and years (Table 4.1). Means were differentiated with Tukey's test for unequal sample sizes (Tukey, 1953, Kramer, 1956, both cited in Zar, 1984, p. 189).

Table 4.1. Numbers of crops of wheat and barley sampled from seven regions of South Australia in quantitative survey of common root rot in 1988 and 1989. Regions are defined broadly in terms of rainfall and soil type, although each is heterogeneous on a local scale.

Region	Soil types *	Mean rainfall†	Numbers of crops			
			Wheat 1988	Wheat 1989	Barley 1988	Barley 1989
(1) *Upper Eyre Peninsula* between Ceduna, Streaky Bay and Cungena	Brown calcareous earths (Gc1.12 and .22); shallow red-brown sandy soils (Uc6.13)	308 mm (Ceduna)	58	12	16	7
(2) *Eastern Eyre Peninsula* Darke Peak to Mangalo	Sandy alkaline yellow duplex soils (Dy5.43)	402 mm (Cleve)	11	-	4	-
(3) *Mid North* Kadina to Kapunda	Brown calcareous earths (Gc1.12 and .22) hard alkaline red duplex soils (Dr2.23 and .33)	389 mm (Kadina) 494 mm (Kapunda)	7	5	-	-
(4) *Yorke Peninsula* south of Kadina	Red shallow porous loamy soils (Um6.24); hard alkaline red duplex soils (Dr2.23 and 2.33)	504 mm (Maitland)	25	18	8	24
(5) *Lower North* Balaklava - Avon - Mallala	Hard alkaline red duplex soils (Dr2.23 and 2.33); dark calcareous loamy earths Gc1.11; calcareous sands Uc1.11	441 mm (Roseworthy)	20	30	14	10
(6) *Northern Mallee*	Brown calcareous earths (Gc1.12 and .22)	274 mm (Loxton)	5	22	3	9
(7) *Southern Mallee*	Grey-brown calcareous loamy earths (Gc1.12)	390 mm (Lameroo)	-	14	3	11

* Soil types from Wright (1969), using classification method of Northcote *et al.* (1975).

† Measurements are from Bureau of Meteorology stations, shown in parentheses, selected as being central in, and/or typical of their respective regions.

4.2.3. Development of common root rot through the growing season in wheat in the field

Progress of common root rot through the season was measured at six field sites in 1987. The sites were those used in Experiment GxE/1987 (Chapter 6), near Mannum, Sanderston and Balaklava, referred to as Mannum, Sanderston Airstrip, Sanderston Driveway, Royal's, Balaklava 110 and Balaklava 170. Populations of *B. sorokiniana* in the soil were measured in five trials about one month prior to sowing, by soil plating on DR70 medium (Chapter 3). This was done to indicate suitability of the sites for experiments, and sampling was not sufficiently intensive to give accurate estimates of inoculum density. The sixth site was presumed to have high inoculum levels, since, before seeding, extensive rotting by *B. sorokiniana* was found on self-sown seedlings. Disease incidence and severity were scored on 7 occasions, approximately 3 weeks apart, using 5 randomly chosen plants from each of 15 plots of wheat variety Machete (susceptible to common root rot; Chapter 7), which formed the buffer to the experiment. Plants were scored for severity of common root rot as before, except that subcrown internodes which were completely discoloured were classed as 4.

4.3. Results

Common root rot was present in all areas of the cereal belt of South Australia (Figures 4.1 and 4.2).

In the quantitative survey of wheat and barley, disease incidence and severity were high in 1988, but in 1989, incidence was low and symptoms were mild on average (Tables 4.2 and 4.3).

Disease incidence on barley on the Eyre Peninsula in 1988, as measured in the qualitative survey (Figure 4.1), was lower than indicated in the more precise, quantitative survey (Tables 4.2 and 4.3). This was attributed to poor recognition of

common root rot by the Eyre Peninsula survey team in 1988, which was rectified by further training for the subsequent year.

Barley generally had higher disease levels than wheat, but this varied between regions and years, giving rise to significant three-way interaction terms for year, crop and region ($p < 0.05$ for incidence and $p < 0.001$ for severity) (Figures 4.3 and 4.4). In some regions, disease levels were similar on the two crops, while in others, barley had considerably more disease. In 1989, when disease severity generally was low, differences in severity between wheat and barley were much less apparent than for the previous year.

Of the 128 wheat crops sampled in 1988, 109 were identified to variety. Statistical comparison was possible (i.e. there were sufficient numbers of crops) between four individual varieties and two pairs of closely related varieties, in a total of 102 crops. Significant differences were shown by ANOVA among these six groups, for both incidence ($p < 0.05$) (Table 4.4) and severity ($p < 0.01$) (Table 4.5). Crop disease levels reflected variety resistance ratings from field trials (Chapter 6), with substantially less disease in Aroona and its backcross derivative, Schomburgk (respectively moderately resistant and moderately susceptible; Table 6.3) than in varieties rated as susceptible. Although the susceptible varieties did not differ significantly among themselves at the $p < 0.05$ level of significance, a trend was evident for disease incidence and severity to be positively related to susceptibility ratings.

The rate of development of common root rot in 1987 differed considerably between the six sites (Figure 4.5). Incidence and severity rose quite rapidly at Balaklava 110 and Mannum, but did not reach appreciable levels until after 100 days at Royal's and Sanderston Driveway. The other two sites were intermediate in rate of disease progression.

Neither the rate of disease development, nor final disease levels, were closely related to initial soil inoculum density of *B. sorokiniana* (Figure 4.5). In particular, rates at

Mannum, which had the lowest density among the five sites measured, were among the highest, and final incidence and severity there were greater than at any site. Royal's had very low rates of disease development, despite showing seedling blight on self-sown plants before seeding.

Table 4.2. Mean percentage incidence of common root rot (percentage of plants with lesions) in crops of wheat and barley surveyed in 1988 and 1989. Each value shows the percentage of crops in the category.

Year	Crop	Incidence category (%)				Number of crops	Mean incidence (%)*
		0-25	>25-50	>50-75	>75-100		
1988	Wheat	12	27	27	34	128	60.4 c
	Barley	0	10	31	59	49	76.5 d
1989	Wheat	33	49	17	1	100	33.6 a
	Barley	9	48	34	9	65	49.4 b

* Means are significantly different by Tukey's HSD test (unequal sample sizes) ($p < 0.05$) unless followed by same letter.

Table 4.3. Mean severity of common root rot (0 to 3 scale) in crops of wheat and barley surveyed in 1988 and 1989. Each value shows the percentage of crops in the category.

Year	Crop	Severity category						Number of crops	Mean* severity (0 to 3)
		0-0.5	>0.5-1.0	>1.0-1.5	>1.5-2.0	>2.0-2.5	>2.5-3.0		
1988	Wheat	22	32	26	12	5	3	128	1.02 c
	Barley	4	27	35	18	6	10	49	1.39 d
1989	Wheat	99	1	0	0	0	0	100	0.19 a
	Barley	94	6	0	0	0	0	65	0.27 b

* Means are significantly different by Tukey's HSD test (unequal sample sizes) ($p < 0.05$) unless followed by same letter.

Table 4.4. Percentages of crops of different wheat varieties, surveyed in 1988, which fell into four categories of percentage incidence of common root rot (percentage of plants with lesions).

Wheat variety (resistance rating*)	Incidence category (%)				Number of crops	Mean incidence (%) †
	>0 -25	>25 -50	>50 -75	>75 -100		
Schomburgk (MS) ‡						
Aroona (MR)	29	36	29	7	14	41 a
Spear & Dagger (MS)	7	33	33	27	15	64 b
Halberd (S)	13	28	19	41	32	61 b
Warigal (S)		38	25	38	8	66 b
Takari (S)		10	40	50	10	74 b
Machete (S)	9	14	41	36	22	68 b

* Resistance rating from field trials (see Chapter 6), MR = moderately resistant, MS = moderately susceptible, S = susceptible. Varieties are ranked from top for increasing numerical ratings.

† Varieties followed by the same letter are not significantly different by Tukey's HSD test (unequal sample sizes) ($p < 0.05$).

Table 4.5. Percentages of crops of different wheat varieties, surveyed in 1988, which fell into six categories of severity of common root rot.

Wheat variety (resistance rating*)	Severity categories						Number of crops	Mean severity (0 to 3) †
	0-0.5	>0.5-1.0	>1.0-1.5	>1.5-2.0	>2.0-2.5	>2.5-3.0		
Schomburgk (MS) ‡								
Aroona (MR)	50	43	7				14	0.55 a
Spear & Dagger (MS)	20	47	13	20			15	1.01 b
Halberd (S)	22	25	28	16	9		32	1.04 b
Warigal (S)	12.5	25	37.5		25		8	1.18 b
Takari (S)		30	40	20	10		10	1.27 b
Machete (S)	14	23	40	9		14	22	1.29 b

* Resistance rating from field trials (see Chapter 6), MR = moderately resistant, MS = moderately susceptible, S = susceptible. Varieties are ranked from top for increasing numerical ratings.

† Varieties followed by the same letter are not significantly different by Tukey's HSD test (unequal sample sizes) ($p < 0.05$).

Figure 4.1. Percentage of barley crop area of Hundreds in South Australia in which common root rot symptoms were found, in a survey in 1988. Intensity of surveying in a Hundred depended on the area sown to barley relative to other Hundreds. Crops were scored for presence or absence of common root rot and the area of each crop was estimated. Refer to Figure 1.1 for scale and other geographical details.

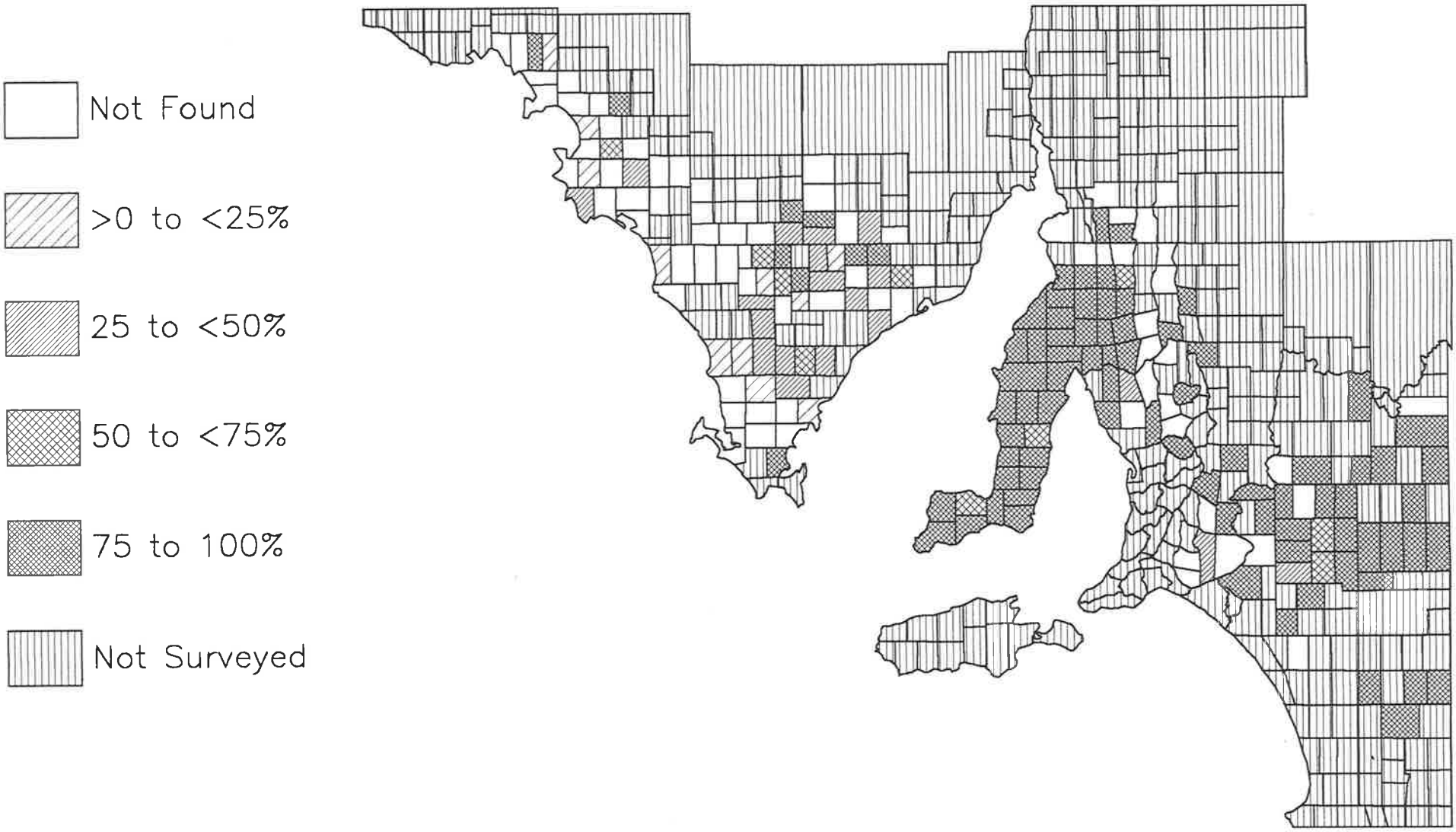

Not Found
>0 to <25%
25 to <50%
50 to <75%
75 to 100%
Not Surveyed

Figure 4.2. Percentage of barley crop area of Hundreds in South Australia in which common root rot symptoms were found, in a survey in 1989. Intensity of surveying in a Hundred depended on the area sown to barley relative to other Hundreds. Crops were scored for presence or absence of common root rot and the area of each crop was estimated. Refer to Figure 1.1 for scale and other geographical details.


Not Found
>0 to <25%
25 to <50%
50 to <75%
75 to 100%
Not Surveyed

Figure 4.3. Regional means of mean incidence of common root rot (percentage of plants with lesions) in crops of wheat and barley surveyed in seven regions of South Australia in 1988 and 1989. Wheat – stippled bars; barley – hatched bars. Error bars are ± standard error. Sample numbers are shown in Table 4.1.

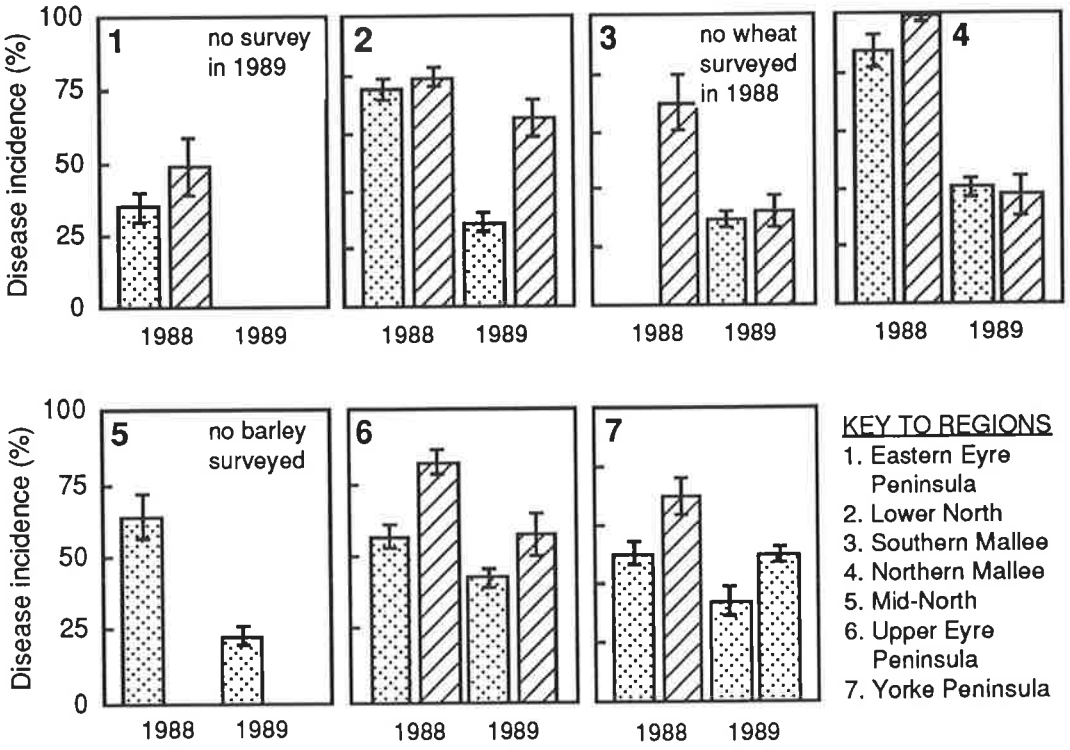

Disease incidence (%)
100
75
50
25
0
1
no survey in 1989
2
3
no wheat surveyed in 1988
4
5
no barley surveyed
6
7
1988
1989
KEY TO REGIONS
1. Eastern Eyre Peninsula
2. Lower North
3. Southern Mallee
4. Northern Mallee
5. Mid-North
6. Upper Eyre Peninsula
7. Yorke Peninsula

Figure 4.4. Regional means of mean severity (0 to 3) of common root rot in crops of wheat and barley surveyed in seven regions of South Australia in 1988 and 1989. Wheat – stippled bars; barley – hatched bars. Error bars are ± standard error. Sample numbers are shown in Table 4.1.

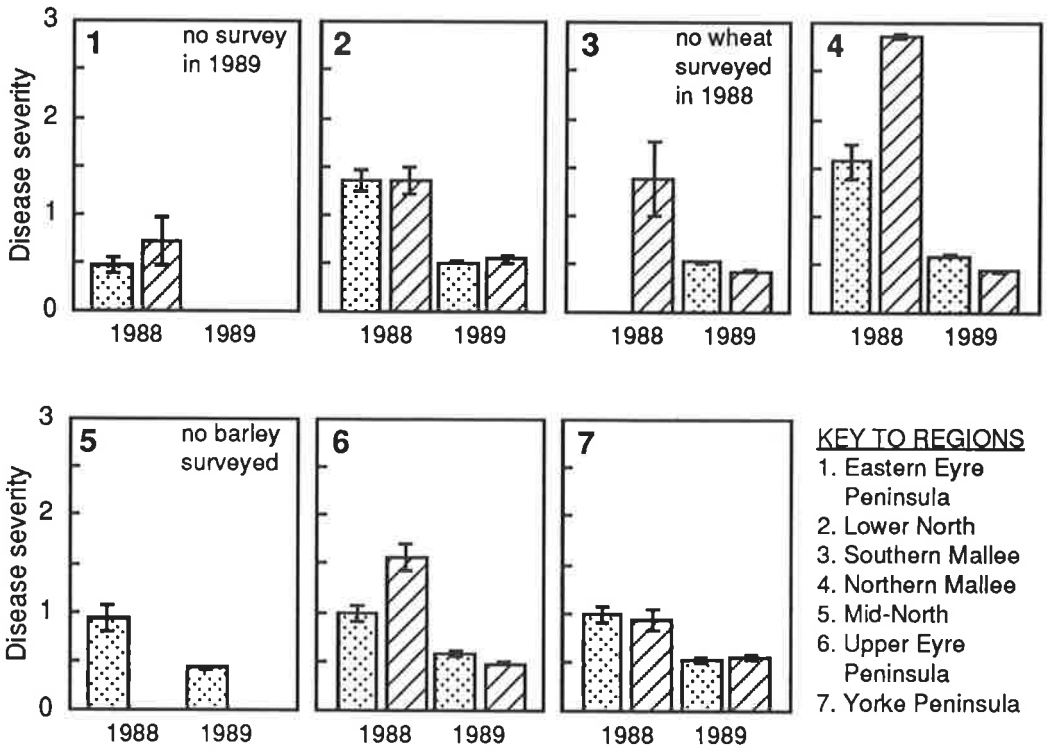
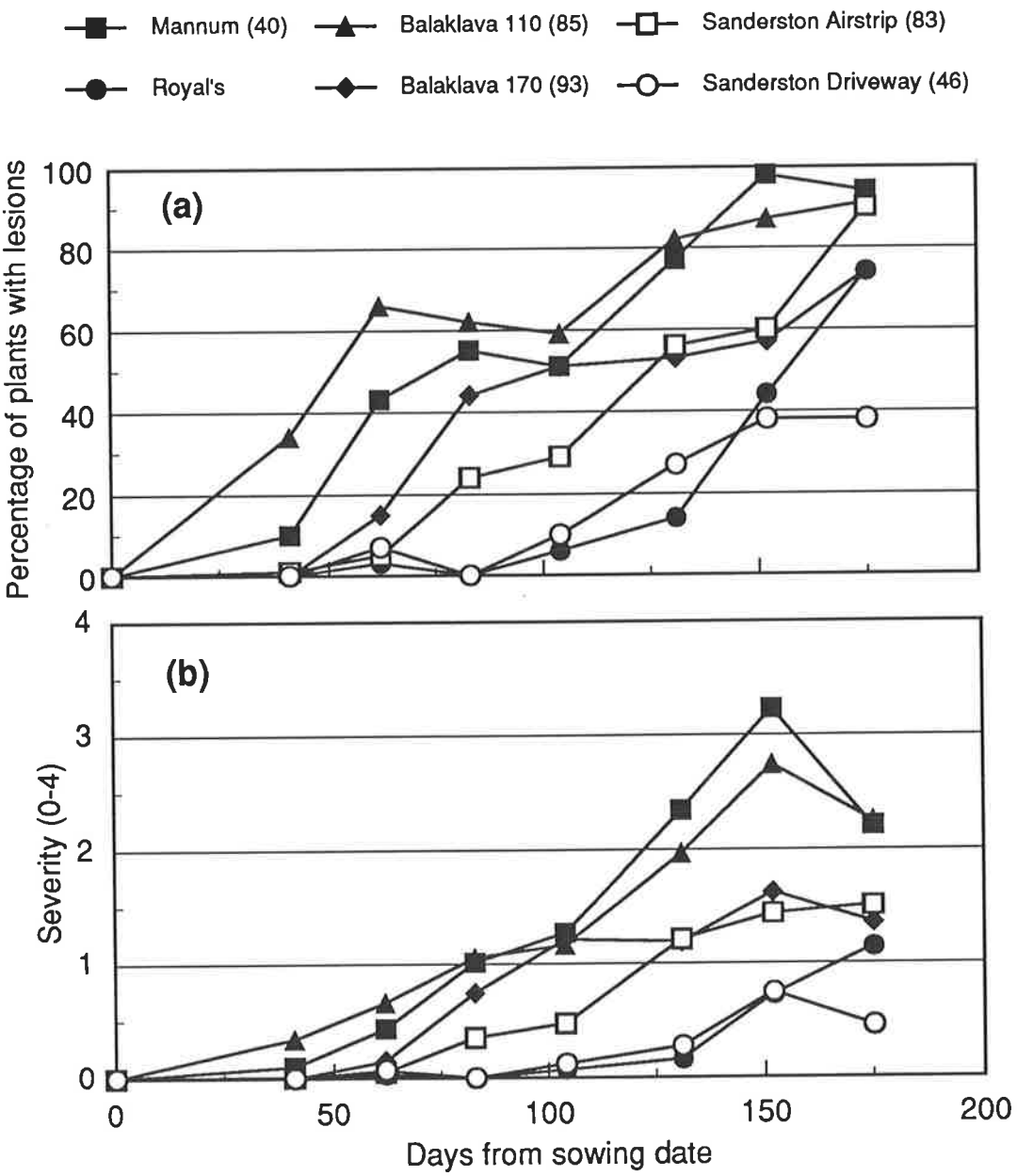

Disease severity
3
2
1
0
1
no survey in 1989
2
3
no wheat surveyed in 1988
4
5
no barley surveyed
6
7
1988
1989
KEY TO REGIONS
1. Eastern Eyre Peninsula
2. Lower North
3. Southern Mallee
4. Northern Mallee
5. Mid-North
6. Upper Eyre Peninsula
7. Yorke Peninsula

Figure 4.5. Development of incidence (a) and severity (b) of common root rot in Machete wheat at six sites in 1987. Inoculum density one month prior to sowing (propagules per gram of soil) is shown, where measured, in parentheses after the site name.


Mannum (40)
Balaklava 110 (85)
Sanderston Airstrip (83)
Royal's
Balaklava 170 (93)
Sanderston Driveway (46)
(a)
Percentage of plants with lesions
100
80
60
40
20
0
(b)
Severity (0-4)
4
3
2
1
0
0
50
100
150
200
Days from sowing date

4.4. Discussion

Common root rot was widespread in South Australia and, in one of two years, was at high levels of incidence and severity in both wheat and barley, across the whole range of rainfall incidence and on all soil types. Incidence of lesions in wheat in 1988 was much higher than those reported from the Canadian Prairies by Machacek (1943), but in 1989, the lower incidence observed was comparable to the Canadian reports. Incidence was also comparable to that reported from Queensland by Wildermuth (1986). The incidence of disease observed in barley in 1988 was comparable to the observations of Piening *et al.* (1976) over three years on the Canadian Prairies.

The widespread distribution of the disease, across varying soils and climatic regions, indicates that the disease is potentially of great economic importance (depending on its effects on yield) not only in South Australia, but also in Western Australia, Victoria and southern New South Wales, where rainfall is also winter-dominant. Except for an unpublished survey from Western Australia (J.M. Wilson, personal communication) the disease has has not been systematically studied in those states. The fact that substantial levels of common root rot did not become evident at some sites until more than half-way through the growing season (about September) (Figure 4.5) confirms that the disease is unlikely to be observed before this time, unless specifically investigated.

Rainfall and temperature data (Bureau of Meteorology) were inspected to explain the variation in disease levels between years. Among 18 recording centres across the state, there was no consistent difference between years in the pattern in rainfall. Temperature, however, differed consistently between the two years, and gives a possible explanation, with 1988 temperatures being more favourable to development of the disease. In North America, common root rot generally does not develop below 15° (McKinney, 1923; Sallans, 1948). Winter temperatures in SA are somewhat below this on average; monthly means for May to October in Adelaide (Kent Town) are 14.4°, 11.7°, 11.0°, 12.6°, 13.8° and 16.6° respectively (Figure 1.2). At the 18

sites in 1988, the months of March through to October had mean daily maxima higher than average, in the 60 or 70 deciles. In 1989, the winter months (June, July and August) were much cooler (deciles 10 or 20, 30 and 30 respectively), while September and October were average or slightly above average (deciles 50 to 60), so still cooler than in 1988. Thus, disease development during the winter and spring of 1988 would have been enhanced by the relatively high temperatures, whereas the cold conditions of 1989 would have retarded development.

Adelaide temperatures are indicative of temperatures elsewhere in the state, although the Mallee and Upper Eyre Peninsula are about 1.5° to 3.0° warmer. Hence, in the average year in many regions, little development of common root rot would be expected in the months June to September. This was demonstrated in the study of common root rot development at six sites in 1987 (Figure 4.5). However, crops sown in mid-May, when soils are still warm, might be expected to develop higher levels of seedling infection than later-sown crops. Casual observations, comparing self-sown plants and the later-sown crop, supported this hypothesis; seedling blight occurred on the former, while common root rot tended to be scarce on the latter. In many areas, crops are sown in mid-May when there has been adequate early rainfall (Taylor, 1991), and in years when early rains are good, higher levels of common root rot might be expected across the state.

The effect of temperature on development of common root rot in South Australia may be compared with that in Canada or other places, by expression of temperatures as growing degree days (GDD), calculated:

$$GDD = \Sigma(\text{mean daily maximum temperature}—\text{base temperature for growth})$$

Bailey (1987) calculated GDD for Saskatoon, Canada using a base temperature of 5°, that of growth of wheat (although 15° might be more suitable for common root rot). In Saskatoon, the growing season of spring wheat is about 100 days (anon., 1987), whereas in South Australia, it is about 170 days (Taylor, 1991). In Saskatoon, common root rot incidence rises rapidly in the first third of the season, to its

maximum after about 30 days (Bailey, 1987), when about 450 GDD have accumulated. The same accumulation does not occur in South Australia (Kent Town) (Figure 4.6) until around the 45th day (if sown 1 June). The total heat unit accumulation in Saskatoon (about 1300 GDD) is reached in South Australia in late September, after about 120 days (20 days longer), with about 50 days remaining until harvest. The total heat accumulation in South Australia is about 2500 GDD. The differences in heat accumulation between South Australia and Saskatoon were parallel with the different shapes of the disease development curves observed in this study (Figure 4.5) and by Bailey *et al.* (1989), as well as the North American studies of Verma *et al.* (1974), Stack (1980), and Verma (1982) in which development was much faster.

The different rates of disease development observed at six sites in 1987 (Figure 4.5) indicate that, while temperature may be important in the development of common root rot on a gross scale, other factors may be more important on an inter-site basis (or in determining disease levels across the state in any one season). Site weather records were not collected, and official recording stations are too few to provide indications, but it is unlikely that the six sites differed substantially in temperature. Disease levels at the six sites were not clearly related to initial inoculum density, but it is unlikely that measurements of inoculum were sufficiently accurate to provide reliable estimates; Wildermuth (1986) found that average common root rot levels in regions were related to inoculum levels in the regions, and Wildermuth and McNamara (1991) showed that severity of common root rot in wheat may be lower after some alternate crops which reduced inoculum levels of *B. sorokiniana*. Bailey *et al.* (1989) found that, while temperature (among several environmental variables) was most closely related with disease development at Saskatoon, precipitation was an important determinant of disease development when water was limiting. Water availability is likely to have varied between the six sites, and to have been limiting to plant growth at times, and may have been important. Also, nutritional and microbiological factors, differing between the sites, may have affected disease development.

Temperature may be partially responsible for regions differing in levels of common root rot (Figures 4.3 and 4.4). For example, the Northern Mallee is warmer than Yorke Peninsula during the day (night time temperatures are similar or lower) and had higher levels of common root rot in both years. It is not possible to draw further inferences in this respect, due to other differences between regions which could affect common root rot. One such difference concerns the cropping regimes into which barley and wheat are sown; the regimes vary in risk of common root rot (Chapter 7). Regions contain several soil types, and some are sown predominantly to barley. These "barley soils" comprise a greater proportion of some regions than others, for example, the coastal lands near Streaky Bay (calcareous sand), appeared to have very high disease levels. On the other hand, wheat may be sown into situations of possibly lower risk of common root rot; it is commonly sown following grain legumes including peas or a grassy medic pasture (shown in Chapter 7 to lead to low inoculum levels), whereas barley is sown after wheat, into higher inoculum densities. Whether the higher disease levels apparent on "barley soils" were due to the soil type *per se*, or to high inoculum levels resulting from regular barley cultivation cannot be resolved on the evidence available.

The relatively higher incidence on barley than wheat was consistent in the two years in North Mallee, Upper Eyre and Yorke Peninsula, but not in the Lower North. This may have resulted from variations to survey routes for the second year, to cover other areas of interest, possibly altering the balance of soil types and rotations.

One explanation for the significant interaction of crop and region concerns the relative susceptibilities to common root rot of the crop varieties grown. Susceptible wheat varieties comprise a greater proportion of the total wheat crop in some regions, bringing the mean susceptibility of the crop closer to that of barley than in regions where less susceptible varieties dominate the wheat crop. For example, most of the Schomburgk (moderately susceptible) and Aroona (moderately resistant) crops are on the Eyre Peninsula, especially Upper Eyre Peninsula, while Machete (susceptible) is particularly common on Yorke Peninsula and the lower North, and Machete and

Halberd (susceptible) are common in the northern Mallee (A.J. Rathjen, personal communication). In the surveyed crops, only Aroona and Schomburgk, comprising 14 % of the crops sampled, could have created the proposed effect, since the other varieties were not significantly different from each other, but the data did not reveal concentrations of these varieties in particular areas.

Rotations also vary between regions, with crops of grain legumes such as field peas (*Pisum sativum*) and faba beans (*Vicia faba*), and sown medic pastures being common on Yorke Peninsula, the mid-North and parts of the southern Mallee, but rare in the drier areas of the northern Mallee, the lower North and Upper Eyre Peninsula. These crops may reduce inoculum of *B. sorokiniana* (see Chapter 7) and thereby reduce disease levels on subsequent cereal crops. Since the standard rotational sequence is legume-wheat-barley, any disease-reducing effect may be reflected in wheat and not barley. There were indications in the survey data (Figures 4.3 and 4.4) that common root rot incidence and severity were lower in the mid-North and Yorke Peninsula than in the drier regions, although the Upper Eyre Peninsula was an exception.

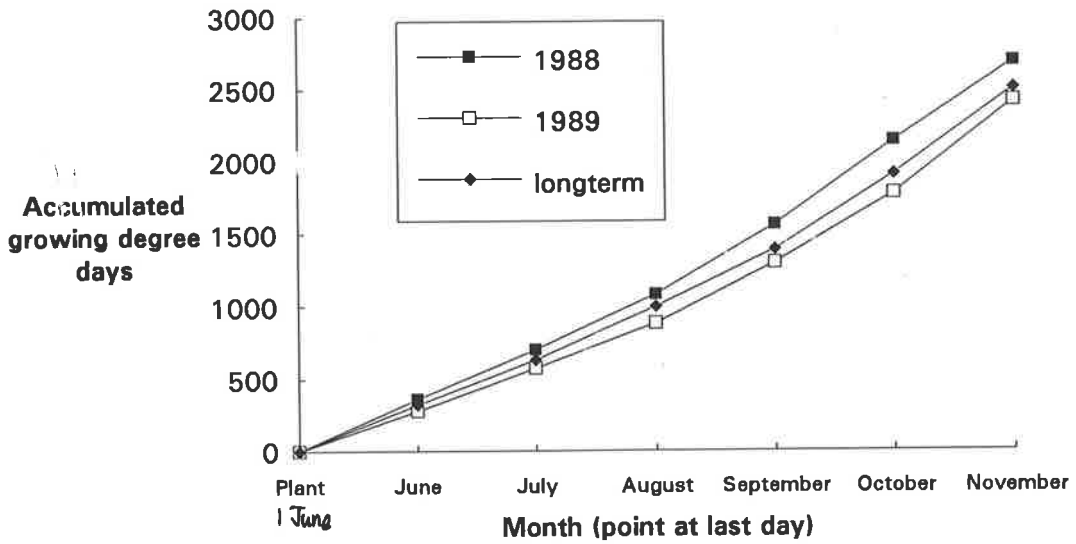
The fact that disease levels on wheat varieties (Tables 4.4 and 4.5) were related to resistance ratings is an important result, (although it should be expected), in that it verified experimental results under farm conditions. It indicates that a substantial reduction in overall disease incidence and severity could be achieved through selection for common root rot resistance, to the level of resistance present among existing varieties. As demonstrated in Chapter 6, some advanced breeding lines are more resistant than Aroona, but others are as susceptible as Machete. Other reports of common root rot surveys have not broken data down into varieties, and the technique of glutenin analyis used here could be applied elsewhere.

Crops with disease levels which were not typical of the variety (e.g. Machete crops with low disease levels or Schomburgk with high disease (Tables 4.4 and 4.5) may have been sown into paddocks with unusually low or high densities of inoculum of *B. sorokiniana*.

Figure 4.6. Accumulated growing degree days (GDD) at Adelaide (Kent Town), South Australia in 1988, 1989 and the long term average (Bureau of Meteorology).

$$GDD = \sum (\text{mean daily max. temperature} - \text{base temperature for growth})$$

where base temperature is taken as 5°.


3000
2500
2000
1500
1000
500
0
Accumulated growing degree days
1988
1989
longterm
Plant 1 June
June
July
August
September
October
November
Month (point at last day)

5. ROLE OF *B. SOROKINIANA* IN COMMON ROOT ROT

5.1. Introduction

B. sorokiniana in South Australian wheat crops was first reported by Samuel (1924). Tinline (1984) showed that this fungus was distributed widely in the state at soil inoculum densities comparable to those in Canada (Chinn *et al.*, 1960; Duczek, 1981) and Queensland (Wildermuth, 1986). It has often been isolated from common root rot-affected tissues in South Australia (Fedel-Moen and Harris, 1987).

The ability of *B. sorokiniana* to cause common root rot as a primary pathogen has been well established, according to Simmonds (1939), who reviewed such studies in North America, and Hynes (1935, 1938) who conducted studies in Australia. However, tissues affected by common root rot are frequently infected by other fungi in addition to *B. sorokiniana* (see Section 2.3), and in some cases, the disease has been regarded as a complex (Greaney *et al.*, 1938).

Debate on these co-infections has led, in South Australia, to the role of *B. sorokiniana* being regarded as unclear. Butler (1961) discussed extensively the range of fungi isolated from affected tissues. Rovira (1980) suggested that *B. sorokiniana* was a secondary invader of tissues already affected by *G. graminis* var. *tritici*, but did not clarify whether it also caused disease independently. Tinline (1984), after finding *B. sorokiniana* and common root rot to be widely distributed in South Australia, argued the fungus to be "at least partly" the cause of common root rot in the state, leaving open the possibility of other causes. Fedel-Moen and Harris (1987) found that *Fusarium* spp. dominated isolates from field-grown wheat roots, and in pathogenicity tests on wheat under artificial conditions, greater damage resulted from inoculations with combinations of these fungi, including *B. sorokiniana*, than from the latter fungus alone. They went so far as to argue for the use of the term "dryland root rot" except for cases in which the principal cause of damage was shown to be *B. sorokiniana*.

Clarification of the cause of a disease is fundamental in determining its economic importance, since agronomic strategies which are effective in controlling disease caused by one fungus or a form of a disease complex may be ineffective with a different pathogen or form of the complex. For example, Tinline et al. (1989) considered that infection by *F. culmorum* may complicate selection for resistance to common root rot.

This chapter reports studies undertaken to clarify the role of *B. sorokiniana* in common root rot in South Australia, using Koch's Postulates. Firstly, a survey was conducted across the state of the occurrence of the fungus in wheat tissues affected by common root rot, expanding the preliminary survey of Tinline (1984) and over a wider area than the studies of Fedel-Moen and Harris (1987). This survey was then reinforced by an investigation of the relationship between common root rot severity and isolation frequency of *B. sorokiniana*, using subcrown internodes of wheat grown in the field. Finally, an experiment was conducted to test the pathogenicity of South Australian isolates of *B. sorokiniana* under controlled conditions simulating the South Australian cropping environment.

The investigations did not include study of other fungi colonising common root rot-affected tissues. Different definitions may be used for the term "disease complex". The broad definition, as applied by Rovira (1980), that co-infecting or successively invading fungi constitute a complex, probably has little practical significance where it is demonstrated that one of the fungi, which has the ability to solely cause the disease, is widely distributed, especially if it is found that control measures, such as examined elsewhere in this thesis, are widely efficacious. To apply the more rigorous definition used by Zogg (1950), that the fungi infecting together interact significantly to cause a disease index higher than that from the strongest pathogen, relies on an elaborate hypothesis, and requires conclusive supporting evidence, to prevail over the simple hypothesis adopted in the studies reported here. The approach taken was to determine the distribution of *B. sorokiniana* and its ability as a primary common root rot

pathogen, and to thereby predict the likelihood that any one instance of the disease in a crop was caused by that fungus, as done in less explicit terms by Tinline (1984).

5.2. Experiments

5.2.1. Survey of wheat crops for *B. sorokiniana*

5.2.1.1. Materials and methods

Selective isolation of *B. sorokiniana* was undertaken on samples from 115 of the wheat crops surveyed in 1988 (see Section 4.2.2). About 20 lesioned subcrown internodes were dissected from each sample, independent of common root rot level, placed in a plastic vial, one vial per crop, and kept at room temperature until plating out, 3 months later.

Subcrown internodes were surface-sterilised, then pushed as far as possible into DR70 agar (in many cases the ends protruded) in a grid arrangement, one sample per Petri dish. Where lesions could be discerned through the semi-opaque medium, marks were made on the base of the dish to locate the lesions for later reference. Dishes were incubated at 25° in darkness for 7 to 8 days, then examined using a dissecting microscope. Fungi identified as *B. sorokiniana* were subcultured for confirmation. The tissue nearest to the centre of the colony was assumed to be the colony's origin. Where such a decision was possible, a record was made of whether the colony had originated from lesioned or unlesioned tissue.

5.2.1.2. Results

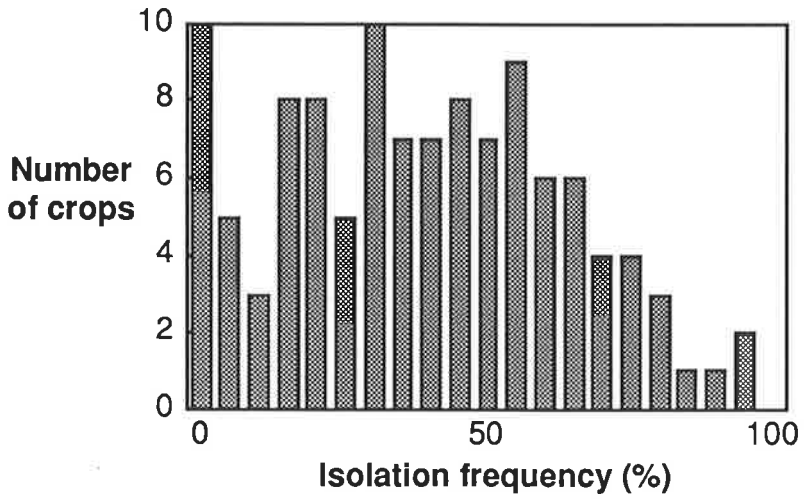
B. sorokiniana was isolated from the lesioned subcrown internodes of 105 of the 115 crops tested (Figure 5.1), at a mean isolation frequency of 42 ± 2.3% (standard deviation) of all subcrown internodes.

Colonies of *B. sorokiniana* generally grew from lesioned tissue, but sometimes developed from apparently healthy tissue, and many badly lesioned sections gave rise to no colony. Many colonies had no discrete origin, but grew from the whole of a

subcrown internode. It was not possible to quantitatively assess the association between colonies and lesions due to several factors. Firstly, difficulties were experienced in locating lesions through the medium (see above). Secondly, by the time sporulation had occurred to enable positive identification of *B. sorokiniana*, colonies often had grown along the subcrown internode. Thirdly, some other fast-growing fungi, particularly *Alternaria* sp. and *Embellisia* sp., often obscured the subcrown internodes before *B. sorokiniana* had grown sufficiently to score. This occurred particularly where tissue protruded from the selective medium, allowing other fungi to escape high concentrations of the selective agents of the DR70 medium.

While the isolation approach used, of attempting to embed tissue in the selective medium, was an improvement over placing tissue on top of the medium, it required further development in order to be useful for quantitation of the association of *B. sorokiniana* with common root rot symptoms. Further development of the plating method is reported below (Section 5.2.2.1).

Figure 5.1. Frequency of isolation of *B. sorokiniana* from lesioned subcrown internodes from 115 crops of wheat surveyed in South Australia in 1988. Mean isolation frequency = 42 ± 2.3% standard deviation.


Number of crops
10
8
6
4
2
0
0
50
100
Isolation frequency (%)

5.2.2. Relationship between severity of common root rot and isolation frequency of *B. sorokiniana* on field-grown wheat

5.2.2.1. Materials and methods

Subcrown internodes were obtained from Machete wheat plants forming a buffer to a fungicide experiment conducted at Windsor in 1989 (see Section 8.3.4). On three occasions (61, 77 and 99 days after sowing), 7 to 10 randomly chosen plants were removed from each of the 15 buffer plots. Subcrown internodes were removed and scored in the laboratory for common root rot severity, then washed, surface sterilised, and plated for isolation of *B. sorokiniana*, keeping internodes grouped by disease severity. Only a subsample of the internodes scored as 0 were plated, whereas all of the diseased internodes were used, since the asymptomatic category dominated.

The method used for isolation was developed for this test, and was called the "water agar/DR70 sandwich" method. This was based on the concept described in Section 5.2.1.1, in which subcrown internodes were pushed as far as possible into the selective agar medium, giving better selectivity than obtained by plating on the surface of the medium. In the sandwich, a layer of quarter-strength water agar (5 g l^{-1}) was poured, and subcrown internodes were embedded in this. A layer of DR70 was then poured over the top, completely covering the tissues. Water agar was used for the first layer because, in pilot studies, it gave no less selectivity than when DR70 was used for both layers, and was much easier to prepare. Quarter-strength was preferred to full-strength, because internodes were more easily embedded in it, and integrity of the final sandwich was not reduced.

Contingency table analysis was used to test the null hypothesis that the isolation frequency of *B. sorokiniana* was independent of the severity rating of plated subcrown internodes.

5.2.2.2. Results

Isolation frequencies of *B. sorokiniana* on the three dates were very similar in each of the four classes of disease severity (Table 5.1), so the data from the three dates were pooled for contingency table analysis.

The null hypothesis was rejected ($\chi^2 = 44.52$, $p < 0.001$). Isolation frequency seemed significantly lower in the healthy category (i.e. 0) than in the three diseased categories (i.e. 1, 2 and 3) (Table 5.2). Isolation frequency seemed lower in the severe disease category (3) than in categories 1 and 2. Retesting the null hypothesis for the categories 1, 2 and 3 only confirmed that isolation frequency was lower in the healthy category than in the diseased categories, but did not indicate the diseased categories to differ among themselves, since the $\chi^2 = 4.25$ was not significant.

The mean isolation frequency from all diseased subcrown internodes was 42.9%, almost identical to that from the wider survey of crops reported in Section 5.2.1. The isolation frequency from all subcrown internodes was 28.9%.

The water agar/DR70 sandwich method of plating gave a large improvement over the partial embedding method, since only three colonies of *Embellisia* sp. and no other fungi besides *B. sorokiniana* were observed.

Table 5.1. Percentages of subcrown internodes of Machete wheat from which *B. sorokiniana* was isolated, in each of the four categories of common root rot severity. Samples were taken on three dates in 1989 at Windsor. Numbers of subcrown internodes from which isolations were attempted in each cell are shown in parentheses.

Sampling date (days after sowing)	Severity of common root rot			
	0	1	2	3
61	11 *(44)*	56 *(50)*	25 *(4)*	0 *(1)*
77	9 *(43)*	45 *(33)*	55 *(11)*	13 *(8)*
99	6 *(34)*	35 *(31)*	35 *(23)*	33 *(9)*

Table 5.2. Contingency table testing the null hypothesis that isolation frequency of *B. sorokiniana* from subcrown internodes was independent of common root rot severity (scale 0 to 3), on Machete wheat grown in the field at Windsor, 1989.
All four classes, $\chi^2 = 44.52 < \chi^2_{0.05,3}$, null hypothesis rejected.
Three diseased classes, $\chi^2 = 4.25 > \chi^2_{0.05,3}$, null hypothesis retained.

	Severity category				Totals
	0	1	2	3	
Colony	11 *(34.9)**	54 *(32.9)*	15 *(11.0)*	4 *(5.2)*	84
No colony	110 *(86.1)*	60 *(81.1)*	23 *(27.0)*	14 *(12.8)*	207
Totals	121	114	38	18	291

* Expected frequencies shown in parentheses

5.2.3. Pathogenicity of *B. sorokiniana* on wheat and barley

5.2.3.1. Materials and methods

The pathogenicity of two isolates of *B. sorokiniana* on wheat and barley was tested in controlled environment, with conditions devised to mimic those aspects of the cropping environment which are important to development of common root rot, as reported in the literature (see Section 2.8)

5.2.3.1.1. Soil

A soil was selected which was low in inoculum density of *B. sorokiniana*, and yet typical in microbial and nutritional characteristics of an agricultural soil. It was obtained from the farm of Mr. B. Heinrich at Coonalpyn on 10 July, 1990. Faba beans (*Vicia faba*) were grown in 1989 and wheat in 1990, planted in May. (In previous years, only low levels of common root rot were found in barley variety trials following grain legume crops on this farm). Soil was taken to a depth of 8 cm from a strip of soil which had not been sown to wheat. Stones and large plant debris were removed by sieving through 1 cm steel mesh. Physical and chemical characteristics of the soil were tested (Table 5.3). The soil was a loamy sand, and was nutritionally suitable for the growth of wheat and barley.

Table 5.3. Physical and chemical characteristics of soil from the Heinrich farm at Coonalpyn, used in test of pathogenicity of *B. sorokiniana* on wheat and barley.

pH	8.4
Electrical conductivity (mS) (tested in H_2O 1:5 v/v)	0.12
Phosphorus ($NaHCO_3$ extract) (ppm)	46
Potassium ($NaHCO_3$ extract) (ppm)	460
Total nitrogen (%)	0.184
Organic carbon (%)	1.67
Sand (%)	87
Silt (%)	1
Clay (%)	12

To determine the drying curve of the Heinrich soil, the procedure of Wallace (1958) was used. Soil was forced through No. 6 mesh (2.80 mm) to remove pebbles and larger plant debris. Soil was placed to 4 cm depth in sintered glass funnels of porosity 4, with a length of water-filled, clear PVC tubing attached to the stem. Water was added to saturate the soil. Suction was varied between funnels by raising or lowering the end of the tube, thus adjusting the distance between the top of the sintered glass plate and the meniscus in the tube (Figure 5.2).

A range of suctions up to 105.5 cm of water was applied, to give the drying curve from saturation to below field capacity (the point at which macropores had drained, so that remaining water was held by capillary action). Covers were placed on funnels and tube ends to minimise evaporation. Soil water was allowed to equilibrate for 24 hours, during which time the meniscus height was adjusted several times to give the desired suction. The soil was removed and weighed, then dried in an oven and weighed again. The gravimetric water content (GWC) was determined as follows and the drying curve plotted (Figure 5.3):

$$GWC\ (\%) = 100 \times \frac{\text{soil weight}_{equilibrium} - \text{soil weight}_{dry}}{\text{soil weight}_{dry}}$$

The drying curve was complex, indicating a range of particle sizes to be present. The soil was saturated between zero and approximately 30 cm of suction, and field capacity was at approximately 45 cm of suction.

The inoculum density of *B. sorokiniana* was determined by soil plating on DR70 medium, to be 33 ± 15 (standard deviation) propagules per gram of dry soil. The soil was bioassayed for other root pathogens which might have caused damage and thereby invalidated the experiment. Seeds of Skiff barley were surface sterilised by soaking for 2 minutes in Milton's solution and washing twice in sterile distilled water. The seeds were pregerminated, then planted 4 cm deep in both the test soil and UC mix (Baker, 1957). They were grown in the glasshouse for 7 weeks at 15 to 20°, and watered as required. Roots were then washed and examined for lesions or deformities. Lesioned sections were washed twice in sterile distilled water, and plated on 1/6 strength NDY agar (Warcup, 1955), supplemented with streptomycin and chlortetracycline (Chapter 3). Growth was adequate in the Heinrich soil, but poor in comparison with the UC mix. There were no significant root disease symptoms, except for small, superficial, brown streaks on the coleoptile on two of 10 plants in the Heinrich soil. Colonies of *B. sorokiniana* grew from these on agar.

Slow-release fertiliser (Osmocote® 120 day release, Sierra Australia Pty. Ltd.) was mixed well into the Heinrich soil at the rate 2.35 g per kg, approximately that recommended for annual plants.

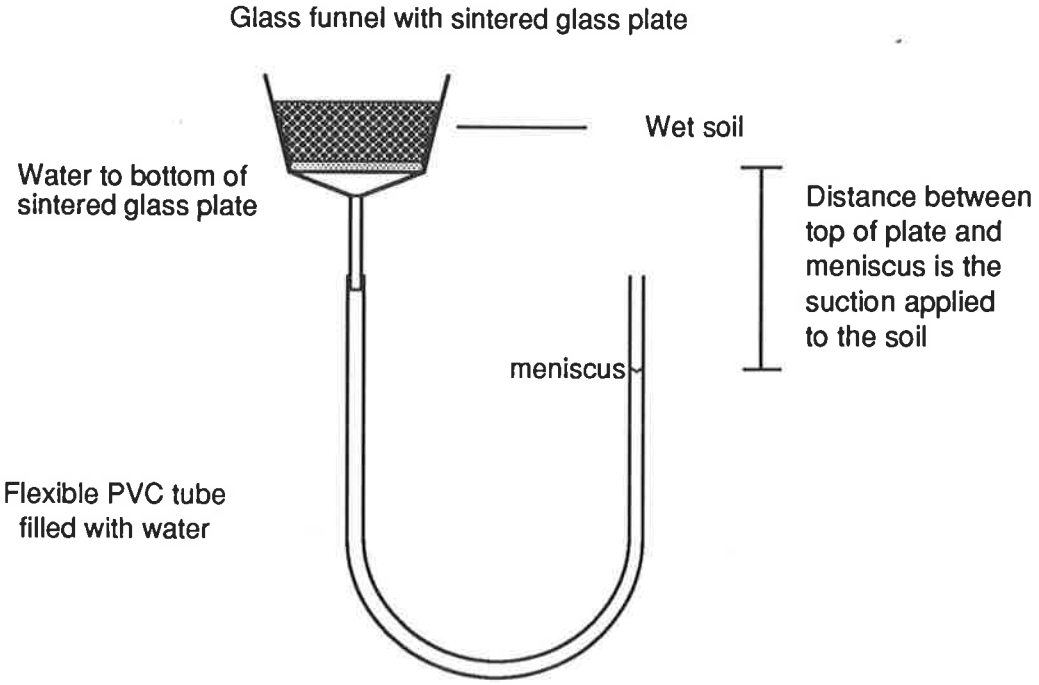
Figure 5.2. Apparatus for determination of soil drying curves (after Wallace, 1958).

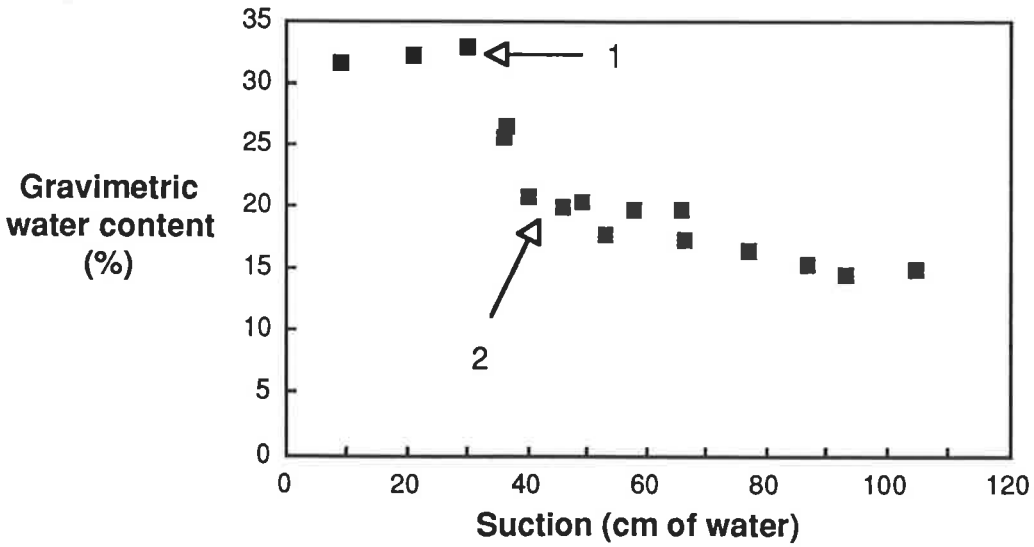
Figure 5.3. Drying curve of soil from the Heinrich farm at Coonalpyn, used in experiment on pathogenicity of *B. sorokiniana* on wheat and barley.

1 - Soil is saturated at lower suctions

2 - Macropores have drained (i.e. field capacity)

Note: 1 bar = 10^5 Pa = 100 KPa = 10m H_20


Glass funnel with sintered glass plate
Wet soil
Water to bottom of sintered glass plate
Distance between top of plate and meniscus is the suction applied to the soil
meniscus
Flexible PVC tube filled with water


Gravimetric water content (%)
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
Suction (cm of water)
1
2

5.2.3.1.2. Construction of apparatus

Although previous studies (McKinney, 1923; Dickson, 1946) indicated that infection by *B. sorokiniana* occurs under a range of soil water potentials, it was considered necessary to devise a means of controlling water potential in a narrow range across the experiment. The commonly used method of watering pots to a predetermined weight was considered unsatisfactory, because, in a sandy soil, water potential changes rapidly with a small change in percentage water content (Figure 5.3), so that large differences in water potential could occur between treatments. Another common method, placing tubes/bottomless pots together on a large soil bed, would have overcome inter-treatment differences, but may still result in large fluctuations in water potential, from field capacity to wilting point, making it difficult to repeat the experiment.

A modification of the method of Liddell and Burgess (1988), who examined the effects of water potential on infection of wheat seedlings by *Fusarium graminearum*, was used. In that system, soil was packed into open-ended tubes, which were placed on a bed of sand, which had been graded by particle size so that it would remain saturated at the desired water potential and would therefore maintain water conductivity at a sufficient level to maintain the head. Water was supplied to the sand with multiple siphons fixed to the base of the sandbed, and water potential, which is directly proportional to the height of the siphon head, was varied by repositioning the reservoir.

A configuration was devised to allow sufficient water conductivity to maintain the head under the higher transpiration load of maturing plants (Figure 5.4).

The material used for "sandbeds" was glass beads (Ballotini Impact Spheres, AE grade, 90 to 150 μm diameter range) which can be substituted for fine sand (C. Hignett, personal communication). The drying curve of the beads was determined (Figure 5.5). The beads drained slowly to about 55 cm, and then more rapidly,

indicating that they would remain nearly saturated at a head of 45 cm, the approximate field capacity of the Heinrich soil. The water conductivity of the beads was high, due to their very smooth surfaces.

Ten plastic crates (Nally No. 5, dimensions 44 cm long x 50 cm broad x 10 cm deep) were used for the sand beds. Two PVC tubes, 12 mm diameter and with perforations (0.3 mm diameter) in their lower sides, were laid in the bottom of a crate, parallel to each other. The tubes protruded through holes in each end of the crate, by 10 cm from one end, and 80 cm from the other and the holes in the crate around the tubes were sealed with grommets and resin. At the centre of the crate, the tubes were taped to the base, so that they inclined slightly upwards towards the ends, to prevent them from floating during the preparation of the sandbed, and so that any air in them would move towards either end. The ends of the tubes were plugged. Glass beads (8 kg) were placed in the crate. Distilled water was added, sufficient to turn the beads to a slurry. The bed was placed on a mechanical culture shaker, and agitated to settle the beads around the irrigation tube. When no more bubbles emerged, the bed was set up on a bench. The tubes were filled with water, one tube at a time, as follows. The crate was gently elevated by about 50 mm at the end with the long tubes, and propped up. The long end of a tube was held above its joint with the crate, and its plug removed. Water was poured in slowly, to allow air bubbles to rise in the tube, reach the meniscus and escape. As it filled, the tube was gradually lowered, so that the meniscus did not rise above the slurry surface. This was to avoid formation of cavities in the sandbed, by the jetting of water through the perforations. When the tube was filled, it was capped and raised again, so that any bubbles in the tube rose to the end. If there were bubbles, the tube was again lowered and refilled. This was repeated until there was no more air in the system. The tubes were then placed in a full bucket of water, the top of which was 45 cm below the surface of the sand. The plugs were removed, establishing the siphon. The water commenced to drain from the sandbed, developing equilibrium with the negative pressure of the siphon.

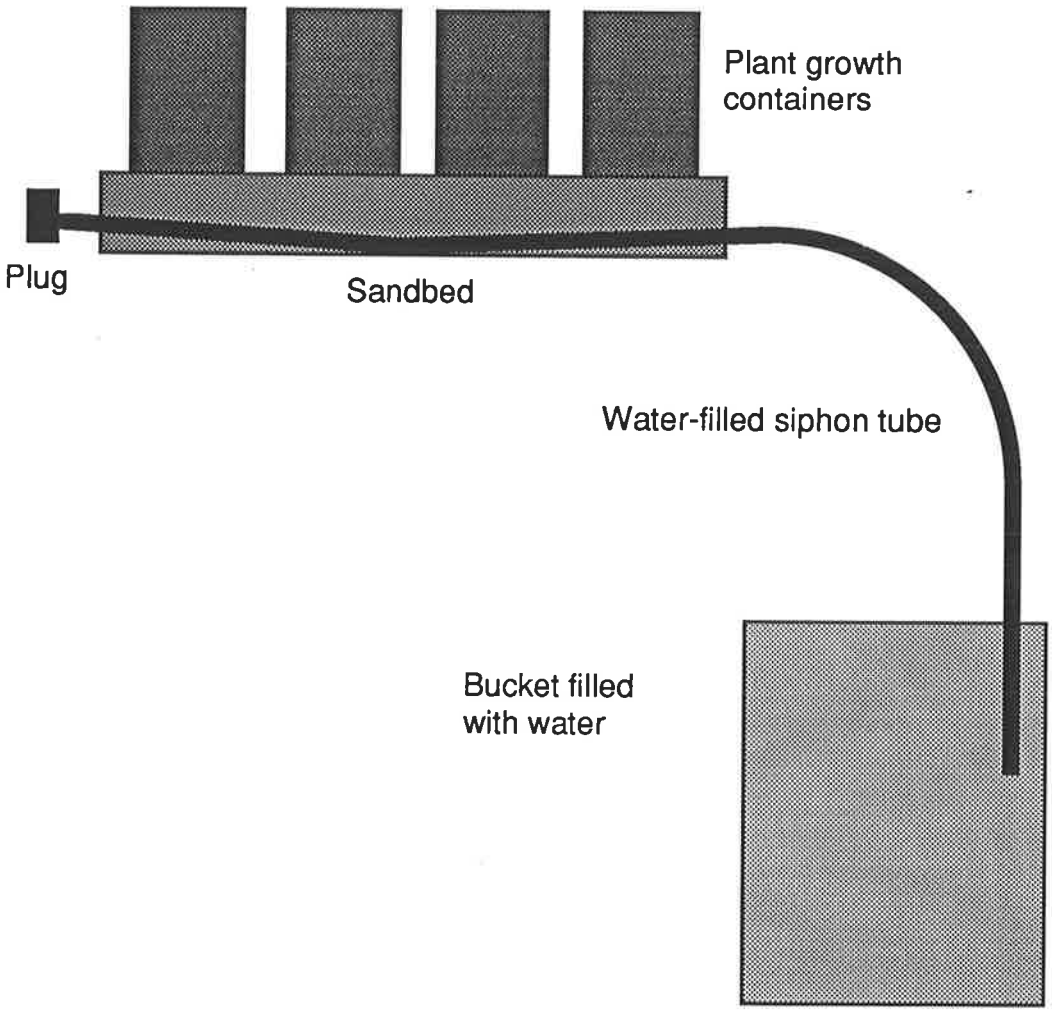
The water columns remained intact for a 48 hour test period. At times during the experiment, when bubbles were observed in the tubes at their high points, the tube-filling process was repeated. If the water column had completely broken, the whole sandbed and pots were resaturated, using a watering can. The tubes were then filled, so that the siphons drained the excess water to re-establish the correct water potential, which took 12 to 24 hours. This was needed more and more frequently as the experiment progressed, presumably because cavities had formed in the sandbed, and because the high transpirational load of the maturing plants exceeded the water conductivity of the system.

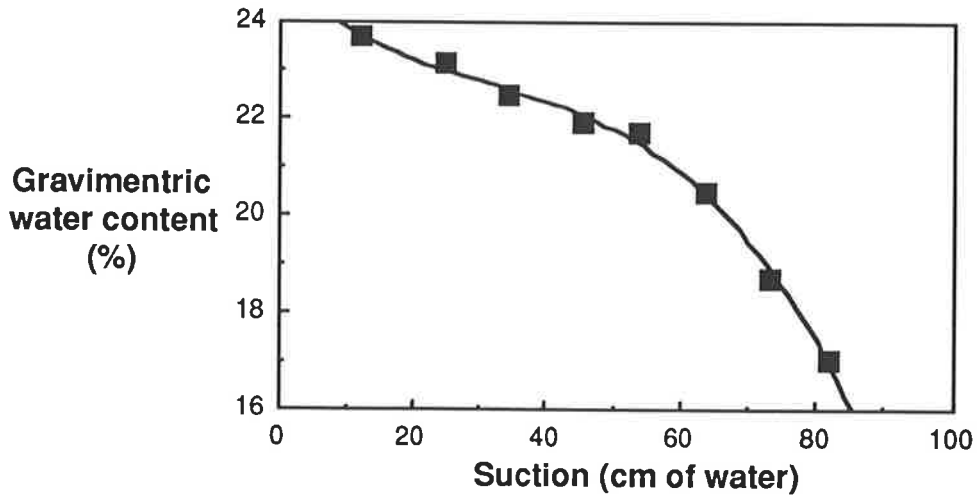
To test the operation of the sandbed, pieces of nylon gauze, 10 cm square, were placed on the sandbed. Five rings of PVC pipe, 85 mm internal diameter and 30 mm height, were placed on the gauze and filled with Heinrich soil. The soil was gently tamped, then saturated with a fine spray. After 48 hours, the gravimetric water content was determined as before. All replicates fitted closely on the drying curve of the Heinrich soil at 45 cm suction (Figure 5.3).

A sheet of gauze was placed on top of each of the ten sandbeds. Cylinders of PVC (85 mm internal diameter, 110 mm height) to act as plant growth containers were stood on this, in a 5 x 3 grid.

Figure 5.4. Apparatus for growing plants at constant soil water potential for experiment on pathogenicity of *B. sorokiniana* on wheat and barley.

Figure 5.5. Drying curve of Ballotini Impact Spheres, used as water-conductive bed in apparatus for maintaining soils at constant water potential in experiment on pathogenicity of *B. sorokiniana* on wheat and barley


Plant growth containers
Plug
Sandbed
Water-filled siphon tube
Bucket filled with water


Gravimentric water content (%)
24
22
20
18
16
0
20
40
60
80
100
Suction (cm of water)

5.2.3.1.3. Experimental design

There were two factors, Species (wheat and barley) and Isolate (wheat isolate, barley isolate and uninoculated) and 20 replicates. Treatments were randomized within replicates. Two replicates were placed in each crate, each in a 2 x 3 arrangement. Between the two replicates in a crate were placed filler rows of three tubes of wheat with the barley isolate, some of which were used through the experiment for monitoring soil water potential and disease progress (see below).

5.2.3.1.4. Inoculation

The two isolates of *B. sorokiniana* were:

- *Wheat isolate* - from a lesioned subcrown internode of variety Halberd from Dublin, South Australia sampled in November, 1989;
- *Barley isolate* - from a lesioned subcrown internode from Long Plains, South Australia sampled in November, 1989.

Cultures were grown on potato dextrose agar at 25° in darkness for 4 weeks, after which they were sporulating densely and had negligible mycelium by bulk. Spore suspensions were made of the two isolates by flooding plates with sterile distilled water, scraping them with a scalpel, decanting them into a 100 ml bottle, and adding a drop of Tween 20. Using a haemocytometer, the concentration of spores in both suspensions was determined to be approximately 90,000 conidia per ml. Soil was inoculated in bulk while slightly damp. Conidial suspensions were diluted 10 times in sterile distilled water, then sprinkled over the soil surface. The soil was thoroughly mixed by turning it repeatedly on a plastic sheet, and sieved again through 2.8 mm mesh. The uninoculated treatment was the base soil, which was also mixed and sieved. Inoculum densities in the three soils were determined by soil plating on DR70 medium , to be 128, 178 and 24 propagules per gram of soil for the wheat isolate, barley isolate and uninoculated base soil respectively.

5.2.3.1.5. Seed, planting and growth conditions

Galleon barley and Machete wheat (both susceptible to common root rot, Chapter 6) were used. Seed from certified pure crops was graded to pass through a 2.8 but not a 2.5 mm sieve, then pregerminated in sterile water in Petri dishes at 20° in darkness and planted two days later.

Base soil (uninoculated) (400 g) was placed in each tube and gently tamped, so that the base of a soil column was in direct contact with the sandbed. Four seedlings were placed in each pot, then 325 g of treatment soil (inoculated or uninoculated) was added and tamped. After six days, plants were thinned to three per tube, by cutting excess seedlings at the base and dabbing glyphosate herbicide on their stubs.

When a crate was completely sown, it was saturated with a fine spray of reverse osmosis water. Plastic bead mulch was placed on the soil surface and on the exposed nylon gauze covering the sandbed, to 1.5 cm depth. Siphons were then established as described above, with a head of 41 cm at the base of the soil columns, equating to 48 cm at sowing depth.

The experiment was conducted in a growth room under fluorescent light banks, supplemented with incandescent bulbs. The light intensity ranged from 80 to 150 μEinsteins $m^{-2}s^{-2}$, at different locations in the room, at the top of the tubes. Temperature was varied during the experiment to mimic field conditions (Table 5.4).

Table 5.4. Temperature regime for test of pathogenicity of *B. sorokiniana* on wheat and barley.

Day	Temp (°)	Growth stage (Zadok's scale)
0	12	0
10	15	2 leaves (12)
21	19	shoot + 1-3 tillers (21-23)
33	21	flag leaf extension - ear emergence (41-51)
40	23	ear emergence beginning - flowering beginning (51-61)
45	25	flowering beginning - medium milk (61-75)

5.2.3.1.6. Assessment and analysis

Monitoring was undertaken on days 17, 30 and 40, when the three non-experimental pots located between the replicates in a crate were removed. The bottom 2 cm of soil was removed from each column and gravimetric water content was determined. Roots were then washed out and examined for lesions, and subcrown internodes, coleoptiles and lesioned root segments were surface-sterilised and plated on DR70 medium.

Harvesting commenced at Day 59, when most grain was ripe. For each tube of plants, fertile tillers and grains were counted, and grain, roots and tops (excluding heads) were dried and weighed. Effects of Species and Isolate on yield and disease parameters was analysed by ANOVA. Yield parameters tested were as listed in Table 5.5. Disease parameters tested were common root rot severity and isolation frequency of *B. sorokiniana*.

Subcrown internodes were cut into 8 pieces, and the sections from each tube (3 internodes x 8 pieces) were surface sterilised. The sections were plated using the water agar/DR70 sandwich method (see Section 5.2.2.1). Sections were embedded in the water agar, in 10 cm square Petri dishes, in a grid of 8 by 3. Before pouring the DR70 medium, each segment was scored for common root rot severity (0 to 3) and this score was written on the dish base next to the segment. A mean score was calculated for each tube. DR70 medium was poured over the water agar layer. Plates were incubated at 25° in darkness for 8 days, then colonies were scored on a three-category scale (0 = no colony; 1 = small colony, which stopped growing before reaching another colony; 2 = full-sized colony, which grew until it reached another colony) (Figure 5.6).

The relationship between disease severity and isolation frequency of *B. sorokiniana* was estimated by linear regression analysis. The model

severity = inoculation + isolation frequency + error

was fitted for both factors, Species and Isolate. Treatments were compared for slope and for y-intercept of the regression, using the F-test and if not different, a common regression for all levels of a factor was calculated.

The independence of yield parameters from the two disease parameters was also tested by regression analysis.

On a random selection of dishes, the two scores (severity and colony size) were recorded for each segment and the relationship between these scores for each of the treatments was analysed. The independence of growth of *B. sorokiniana* colonies and common root rot severity score from single subcrown internode segments, was tested. As both variables were ordinal (scored 0 to 2 and 0 to 3 respectively), contingency table analysis was used. The relationship was tested for all levels of both Species and Isolate. No statistical method was available for comparing treatments within factors (T. Hancock, personal communication).

5.2.3.2. Results and discussion

5.2.3.2.1. Monitoring

Gravimetric water content of soil at the base of soil columns (41 to 43 cm of suction) at 17 days was 18.3 ± SD 0.4 %, at 30 days it was 18.1 ± SD 0.5 % and at 40 days it was 17.8 ± SD 0.5 %. These were close to the drying curve of the Heinrich soil (Figure 5.3). That the gravimetric water content was slightly lower than expected could be explained by hysteresis (the difference between drying curves and wetting curves). As the head was regulated by maintaining the reservoir level, which fell by up to 2 cm before being refilled, the water relations in the soil may have moved from the drying curve to the wetting curve. This was insignificant, since the water potential remained close to the objective.

The roots of plants sampled at 17 days showed moderate knotting by cereal cyst nematode (*Heterodera avenae*), which requires temperatures below 15° for hatching and so was favoured by the low temperature in the first 10 days, but not revealed by

the initial bioassay. The damage induced by this pest would have reduced final yields and increased variability.

At 17 days, very small, superficial gold/brown lesions, similar to those observed in the soil bioassay (see Section 5.2.3.1.1) were present on the coleoptiles of plants grown in the base soil. These consistently yielded *B. sorokiniana* colonies on agar. At 30 days, these lesions had developed to slight/moderate severity, covering about 25 % of affected subcrown internodes. Similar lesions were also visible on the epidermis of crown roots, within 10 to 15 mm of their proximal ends (Figure 5.7). *B. sorokiniana* was consistently isolated from lesions on the subcrown internodes and crown roots. At 40 days, the lesions on roots and subcrown internodes had increased in severity, penetrating the cortex. At harvest (day 59 onwards), the proximal 20 to 30 mm of crown roots were severely discoloured dark brown through their entire diameter (Figure 5.8).

5.2.3.2.2. Effects on yield parameters

None of the growth and yield parameters were significantly affected by inoculation, but coefficients of variation for all parameters were high (Table 5.5), giving little chance of obtaining significant differences. Major sources of variability would have been:

- from growth room factors (light intensity, temperature and airflow varied substantially at different locations);
- border effects (some tubes were bordered by crate ends, others by other tubes on all sides).

The border effects were probably the major source of variability, since growth room factors were minimised by blocking replicates. Larger sandbeds, each containing more replicates, could reduce this problem. A Latin square design may have been preferable, as it can account for within-block variability, whereas the randomized complete block design used in this experiment accounts only for between-block variability .

None of the regressions of disease parameters against yield parameters were significant. Only minimal amounts of the high levels of variability were accounted for. Time to maturity (59 days) was probably reduced from normal (170 days in the field) by the low light intensity in the cabinet and the effects of cereal cyst nematode (see Section 5.2.3.2.1).

Table 5.5. Means and coefficients of variation of yield and disease parameters in controlled environment test of pathogenicity of *B. sorokiniana* on wheat and barley.

Parameter	Sample mean	Coefficient of variation (%)
plants per tube	2.95	10.7
heads per tube	6.71	43.2
heads per plant	2.309	45.0
grains per tube	63.4	47.7
grains per head	11.13	67.3
grains per plant	21.74	47.5
yield per plant	1.743	45.8
single grain weight	0.0284	32.7
yield per head	0.285	55.1
yield per plant	0.6	46.8
root weight per plant	1.37	567
yield per g of root	3.57	67.4
grains per g of root	132.9	62.1
common root rot severity	2.174	30.0
Isolation frequency of *B. sorokiniana*	0.786	34.0

5.2.3.2.3. Effects on common root rot

The interaction term of Species x Inoculation was significant ($p < 0.05$) (Table 5.6) although no interactions were distinguished by Tukey's HSD test. The interaction appeared to arise from severity being lower on barley than on wheat in the uninoculated soil, (due to the background inoculum which was clearly favoured by the experimental conditions), whereas the two species had similar severities in the two inoculated treatments. This was probably due to difficulties experienced in scoring barley. On wheat, the discolouration was definitive. Segments classified as 0 were clean white. Lesions were distinct even when small, and when large, were very dark brown. Symptoms were less clear on barley. Many segments were uniformly slightly

discoloured, and it was difficult to classify them as either 0 or 1. In the uninoculated treatment, there were many more segments with low disease, on which scoring was complicated. Hence, the problem was only significant in the uninoculated treatment.

5.2.3.2.4. Effects on isolation frequency of *B. sorokiniana*

Frequency of isolation of *B. sorokiniana* from subcrown internode segments was significantly higher by 14.3% in wheat than in barley ($p < 0.001$, Table 5.7), reflecting severity scores.

Isolation frequency was significantly lower in the uninoculated treatment than in the two inoculated ones, which were nearly equal ($p < 0.001$).

The marginal significance of the interaction term in common root rot severity was not repeated in isolation frequency, although similar numerical differences occurred (much lower isolation frequency from barley than from wheat in the uninoculated soil).

Figure 5.6. Water agar/DR70 sandwich isolation plates, showing layout of samples. Columns are all segments from a single subcrown internode. Each plate held six subcrown internodes, three from a treatment (grouped together). Black colonies are *B. sorokiniana*. Short, horizontal lines are subcrown internode segments from which no colony grew. Numbers written by these are severity scores. All colonies on plates shown were scored as "full size" (score 2). No "small" colonies are present, but these were about half the diameter of standard colonies, whereas full-sized colonies were defined as those which grew until restricted by adjacent colonies. Plate dimensions 10 cm x 10 cm.

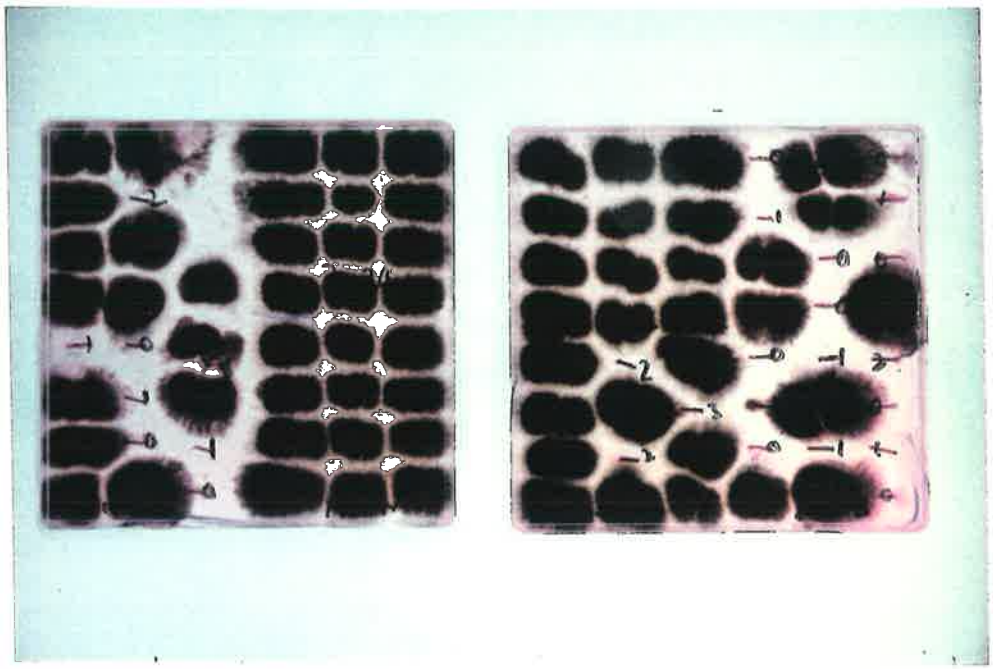


Figure 5.7. Superficial, elongate epidermal lesions (arrowed) on crown roots and tiller bases of wheat plants grown in field soil infested with *B. sorokiniana*, 30 days after planting. This fungus was consistently isolated from the lesions (see Section 5.2.3.2.1). Scale 8:1.

Figure 5.8. Severe, dark brown necrosis of crowns, crown roots, coleoptiles and subcrown internodes of wheat plants grown in field soil infested with *B. sorokiniana*, 59 days after planting (see Section 5.2.3.2.1). Scale 1.6:1.

Table 5.6. Mean severity of common root rot (scale 0 to 3) on wheat and barley after inoculation with isolates of *B. sorokiniana* from wheat and barley. The analysis was performed on severity data which were the means of all 24 segments from the three plants in a single growing tube.

ISOLATE	SPECIES		Isolate mean
	Wheat	Barley	
Wheat isolate	§2.53 c	2.23 bc	2.38
Barley isolate	2.42 bc	2.48 bc	2.45
Uninoculated	1.93 ab	1.46 a	1.69
Species mean	2.29	2.06	2.17

§ Species x Isolate term significant at $p < 0.05$. Means followed by same letter are not significantly different by Tukey's HSD.

Table 5.7. Mean percentage isolation frequency of *B. sorokiniana* from wheat and barley after inoculation with isolates from wheat and barley. The analysis was performed on severity data which were the means of all 24 segments from the three plants in a single growing tube.

ISOLATE	SPECIES		Isolate mean#
	Wheat	Barley	
Wheat isolate	§95.1	82.3	88.7 b
Barley isolate	92.0	90.2	91.1 b
Uninoculated	65.0	48.1	56.5 a
Species mean†	84.0	73.5	78.8

† Species term significant at $p < 0.001$.

Isolate term significant at $p < 0.001$. Means followed by same letter are not significantly different by Tukey's HSD test.

§ Crop x Isolate term not significant.

5.2.3.2.5. Relationship between common root rot severity and isolation frequency of *B. sorokiniana*

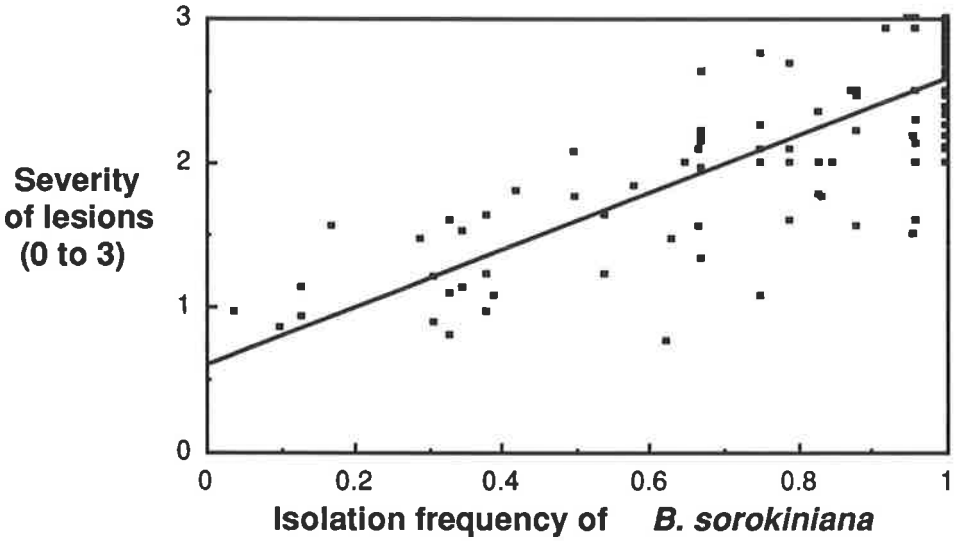
Linear regressions of severity on isolation frequency did not differ between wheat and barley, or between the three inoculation treatments. Consequently, the common regression was calculated (Figure 5.9):

severity = 0.61 + 1.98 x isolation frequency.

This accounted for 66% of the variance, and was highly significant at $p < 0.001$, showing common root rot severity to be closely related to isolation frequency of *B. sorokiniana*.

Isolation frequency from the four categories of disease severity is further discussed in Section 5.2.3.2.6.

Figure 5.9. Linear relationship between severity of common root rot symptoms and isolation frequency of *B. sorokiniana* on subcrown internode segments. Regression severity = 0.61 + 1.98 x isolation frequency, $r^2 = 0.66$, $p < 0.001$.


Severity of lesions (0 to 3)
3
2
1
0
0
0.2
0.4
0.6
0.8
1
Isolation frequency of B. sorokiniana

5.2.3.2.6. Relationship between common root rot severity and colony size of *B. sorokiniana*

For both factors, Species and Isolate, χ^2 statistics for severity versus colony size were highly significant, so the null hypothesis that size of a *B. sorokiniana* colony growing from a subcrown internode segment was independent from the common root rot severity rating of the segment was rejected for both factors (Species, $p < 0.001$, Table 5.8 parts 1 & 2; Isolate, $p < 0.001$, Table 5.8 parts 3, 4 and 5). The strong trends were:

- few colonies grew from segments scored as 0, or healthy;
- small colonies grew from segments with slight to moderate symptoms;
- "full-sized" colonies grew from severely diseased segments;
- isolation frequency of *B. sorokiniana* increased with increasing disease severity.

Table 5.8. Contingency table testing the null hypothesis that severity of common root rot symptoms on a subcrown internode segment was independent from size of *B. sorokiniana* colony growing from that segment. The null hypothesis was tested for the two hosts, wheat (1) and barley (2) (facing page), and the three inoculation treatments, wheat isolate (3), barley isolate (4) and uninoculated (5) on both hosts (overpage).

Observed frequencies are shown in plain type; expected frequencies are shown in italics and parentheses.

1. Wheat (χ^2 = 212.9, H_0 rejected, $p < 0.001$)

Colony growth	Severity 0	1	2	3	Total
0	17 *(2)*	19 *(5)*	10 *(9)*	5 *(35)*	51
1	1 *(2)*	8 *(5)*	18 *(8)*	23 *(35)*	50
2	1 *(15)*	14 *(31)*	43 *(54)*	268 *(226)*	326
Total	19	41	71	296	427
Isolation frequency (%)	10.5	87.8	90.1	98.3	88.1

2. Barley (χ^2 = 127.3, H_0 rejected, $p < 0.001$)

Colony growth	Severity 0	1	2	3	Total
0	15 *(4)*	40 *(15)*	31 *(31)*	12 *(48)*	98
1	2 *(4)*	10 *(16)*	39 *(33)*	53 *(51)*	104
2	1 *(10)*	18 *(36)*	66 *(72)*	145 *(72)*	230
Total	18	68	136	210	432
Isolation frequency (%)	16.6	41.2	77.2	94.3	77.3

Table 5.8 *continued.*

3. Wheat isolate (χ^2 = 163.3, H_0 rejected, $p < 0.001$)

Colony growth	Severity 0	1	2	3	Total
0	4 *(0)*	22 *(5)*	7 *(8)*	0 *(20)*	33
1	0 *(1)*	11 *(10)*	32 *(18)*	28 *(43)*	71
2	0 *(3)*	14 *(33)*	45 *(58)*	177 *(142)*	236
Total	4	47	84	205	340
Isolation frequency (%)	0	53.2	91.7	100	90.3

4. Barley isolate (χ^2 = 64.2, H_0 rejected, $p < 0.001$)

Colony growth	Severity 0	1	2	3	Total
0	6 *(1)*	5 *(1)*	5 *(4)*	6 *(16)*	
1	1 *(2)*	2 *(3)*	15 *(9)*	33 *(37)*	51
2	1 *(6)*	8 *(10)*	23 *(30)*	130 *(117)*	162
Total	8	15	43	169	235
Isolation frequency (%)	25.0	66.7	88.4	96.4	90.6

5. Uninoculated (χ^2 = 123.5, H_0 rejected, $p < 0.001$)

Colony growth	Severity 0	1	2	3	Total
0	30 *(12)*	33 *(18)*	21 *(26)*	5 *(34)*	89
1	1 *(4)*	5 *(6)*	13 *(8)*	10 *(11)*	29
2	2 *(17)*	10 *(25)*	36 *(36)*	78 *(48)*	126
Total	33	48	70	93	244
Isolation frequency (%)	9.1	31.3	70.0	94.6	63.5

5.3. Discussion

The experiments clearly demonstrated, through Koch's Postulates, that *B. sorokiniana* is a major cause of the common root rot symptoms observed across the cereal belt of South Australia at high levels in wheat and barley (Chapter 4). Firstly, it was isolated from subcrown internodes of 105 of 115 wheat crops from across the State's cereal belt (see Section 5.2.1). Secondly, it was more frequently isolated from diseased than asymptomatic subcrown internodes of field-grown wheat (see Section 5.2.2). Thirdly, in a pathogenicity test under laboratory conditions which were devised to mimic as far as practicable the field, inoculation with the fungus resulted in lesions characteristic of, although more severe than, the disease in the field (see Section 5.2.3). Finally, in this pathogenicity test, there was a close association between inoculation with *B. sorokiniana*, the presence and severity of lesions on wheat and barley, and the re-isolation frequency of the fungus and its vigour or inoculum potential.

That *B. sorokiniana* grew from asymptomatic tissue in each of the tests, as previously observed by Harding (1972) and Bailey (1987), are explained by the finding of Huang and Tinline (1976) that the fungus invades tissue before necrosis occurs. More interesting is that, in each of the tests, a proportion of diseased tissue sections (about 57% in Sections 5.2.1 and 5.2.2, and from 0 to 69% in Section 5.2.3) did not give rise to isolates. The simplest hypothesis is that, while *B. sorokiniana* caused the lesions, it was not able to grow for some reason. Presumably through competitive activity by other fungi (Statler and Darlington, 1972; Tinline *et al.*, 1975) and other microorganisms, the nutrients required for growth by *B. sorokiniana* may have been exhausted or unavailable. Isolation frequency of *B. sorokiniana* from diseased tissue was much greater in the pathogenicity test (Section 5.2.3, Table 5.8) than in the two field studies (see Section 5.2.1, Figure 5.1, and Section 5.2.2, Tables 5.1 and 5.2). This was probably due to competition; in the pathogenicity test, competition from other fungi would have been much lower than in the field, since experimental conditions in the pathogenicity test clearly favoured infection by *B. sorokiniana*, leading to rapid infection and severe disease. Even if the fungus remained viable in

the tissue, it may have been unable to obtain replacement nutrients from the DR70 medium, due to the selective agents. Also, the technical difficulties in excluding other fungi in the two field studies may have substantially reduced the estimate of isolation frequency.

Tinline (1984), after a study of the distribution of common root rot and *B. sorokiniana* in South Australia, left open the possibility that this fungus was not the sole cause of the disease. Fedel-Moen and Harris (1987), from their findings that *Fusarium* spp. which were more abundantly isolated from rotted wheat tissues than *B. sorokiniana*, caused more damage to wheat when inoculated in combination with *B. sorokiniana* than when that fungus was inoculated alone, argued against the use of the term "common root rot" in South Australia; this, they proposed, assumed that *B. sorokiniana* was the major pathogen, and they did not accept that assumption. Corroborating their findings, Vanstone *et al.* (1991) found that lesions on subcrown internodes of wheat at two sites in South Australia were correlated with isolation frequency of *Fusarium* spp., but not that of *B. sorokiniana*, which was isolated from 16% of subcrown internodes.

However, the studies of neither Vanstone *et al.* (1991), nor Fedel-Moen and Harris (1987) provide clear evidence on which to reject the simple hypothesis that *B. sorokiniana* alone caused the common rooot rot symptoms, and that the co-infecting fungi were more than opportunistic saprophytes which may have displaced *B. sorokiniana*. The low isolation frequency of *B. sorokiniana* achieved by Vanstone *et al.* (1991) may have been partly determined by technique; they used a non-selective medium, and the studies reported here showed that this may not optimise *B. sorokiniana* isolation frequency. The results of Fedel-Moen and Harris (1987), which relied on frequency of isolations and pathogenicity tests of the co-infecting fungi, are weakened by two problems. Firstly, frequency of isolation of fungi does not constitute evidence for their involvement in production of symptoms associated with infection by another fungus. Secondly, pathogenicity tests have little meaning unless the conditions under which they are conducted mimic cropping conditions, which was

not the case in that study. Combining a fungus with a plant under conditions optimal for the growth of the fungus but detrimental to the plant's growth may result in a level of infection and severity of symptoms not to be observed under cropping conditions. Inoculating a plant with a combination of fungi under atypical conditions may result in a significant interaction between the fungi, although this interaction may be inconsequential in field conditions. Hence, the argument of Fedel-Moen and Harris (1987) that a different name should be used in South Australia for the typical symptoms of common root rot, cannot be supported.

Rovira's (1980) suggestion that the fungus was a secondary invader may have been based on casual observations during studies of other root diseases. Common root rot has generally not been systematically recognised in South Australia, so may have been overlooked in many investigations. Root diseases in that state have tended to be examined in winter or early spring, which is appropriate for cereal cyst nematode (*H. avenae*), take-all (*G. graminis* var. *tritici*) and Rhizoctonia barepatch (*Rhizoctonia solani*), or late in the season in specific investigations of dramatic "haydie" symptoms involving *G. graminis* var. *tritici*. Neither type of investigation will reveal common root rot, since significant severity is not reached until late in the season (Figure 5.2 and Section 4.4), and it does not cause dramatic patches of dead plants. Both common root rot and take-all can cause brown to black discolouration of the subcrown internode, and incorrect diagnoses may be made where fungi are not isolated (Price, 1970; Butler, 1961). [It was observed in the present study that, in plants with black lesions in the steles of primary roots, both *B. sorokiniana* and *G. graminis* var. *tritici* may be associated with subcrown internode lesions, but when no such root lesions were present on the roots, *G. graminis* var. *tritici* was unlikely to be isolated from the subcrown internode].

It is likely that the close relationship between lesion severity and colony size (see Section 5.2.3.2.6) was due to the isolation medium restricting propagules to their existing nutrient base or inoculum potential. The base medium of DR70 agar is starch nitrate agar (SNA). *B. sorokiniana* will grow from a point inoculation on SNA to

completely cover a plate, presumably by exploiting the nutritive potential of the medium. However, colonies on DR70 agar always remain restricted, either in soil or tissue plating. Apparently, the medium inhibits the fungus from exploiting nutrients beyond those within the propagule or tissue already colonised. That saprophytic growth does occur on non-restrictive media and untreated plant tissue indicates that the relationship observed (colony growth dependant on symptom severity) may not have practical significance for the field. However, it remains an important part of the demonstration of pathogenicity of *B. sorokiniana* through Koch's Postulates.

The water agar/DR70 sandwich developed in these studies was powerful and has wide application in work on *B. sorokiniana*, and a similar approach could be used with appropriate media for other host/pathogen systems. Firstly, it was much more selective than placing sections on top of the agar, and may improve isolation frequency. Secondly, because of this increased selectivity and the ability to acurately position sections, more sections than usual could be placed on a plate, allowing more efficient use of time and materials. Thirdly, because lesions could be observed after plating, it was easier to observe colony origin and vigour in relation to lesion location and severity. The ease of plating and the ability to observe lesions after plating enabled the sectioning of individual subcrown internodes, which greatly enhanced the accuracy of scoring of severity and extent of colonisation.

The apparatus for maintaining constant water potential enabled a satisfactory level of control of experimental conditions. Modifications to the system could reduce the difficulty in maintaining the head under higher transpirational loads. A slightly improved system would be very useful in a range of studies on soilborne diseases, on which experiments are often confounded by soil water potential (see Section 2.8.2). A limitation of the system is that the need for high water conductivity restricts its usefulness to above 0.1 bar, approximately, which for some soils is above field capacity.

6. RESISTANCE IN WHEAT AND BARLEY TO COMMON ROOT ROT

6.1. Introduction

Resistance was shown in previous studies to be a promising method for control of common root rot in wheat, since resistant germplasm is available, and genetic improvement can be made by selection on disease ratings (Tinline *et al.*, 1989; see Section 2.9.3). Wildermuth (1974) demonstrated that moderate resistance was present among Australian wheat varieties, and it has been adopted as a selection criterion in wheat breeding in Queensland. Preliminary studies (J. Lewis, personal communication) indicated that some South Australian varieties may have moderate resistance.

Tinline and Ledingham (1979) observed that, in a set of wheat lines, there was a close correlation between the level of common root rot on the line, and yield reduction from the disease. This indicates that genetic improvement in resistance could be achieved both by selection on disease ratings, and on yield where common root rot was present. The South Australian breeding programs would permit both; an F_2 progeny method is used, in which F_2-derived lines are selected at the F_2 and the F_6 on the basis of yield under commercial conditions, and at other times for disease resistance in nurseries (Rathjen and Pederson, 1986). Selection for common root rot resistance under this system would be more efficient than in a program based on the pedigree method, unless common root rot resistance was a major selection criterion and was precisely assessed and intensively selected (Whan *et al.*, 1982). In a genetic model involving three or more genes, as may be the case for common root rot (Sallans and Tinline, 1965; McKenzie and Atkinson, 1968; Larson and Atkinson, 1981; Stack, 1982; Bailey *et al.*, 1988), the frequency of genotypes in the F_2 and its progeny with the desired allelles is greater than at the homozygous stage, so that fewer lines need to be screened than with bulk and single seed descent methods (Whan *et al.*, 1982).

In barley, the range of resistance identified is narrower (Cohen *et al.*, 1969; Piening *et al.*, 1969; Tinline and Ledingham, 1979; Duczek, 1984; Grey and Mathre, 1984; Stack, 1986) and the modes of inheritance are not known (Cohen *et al.*, 1969). Also, there is a less clear relationship between variety ratings and yield loss in barley than in wheat (Piening, 1973; Tinline and Ledingham, 1979), so that selection on yield in the presence of common root rot will not select for resistance. However, some studies have indicated consistent varietal rankings within the narrow range (Duczek, 1984; Grey and Mathre, 1984; Stack, 1986), so that selection on disease levels may be feasible.

The studies reported here were undertaken to determine the range of resistance to common root rot available in the South Australian wheat and barley breeding programs, to allow South Australian breeders to make informed decisions on whether resistance to common root rot should be adopted as a breeding objective in their programs, what strategies and methodology would be appropriate, and whether further research on resistance is required or warranted.

6.2. Materials and methods

6.2.1. Field trials

A set of experiments was performed from 1984 to 1988, in which varieties and breeders' lines of wheat and barley were sown in field plots, with natural inoculum of *B. sorokiniana*, to screen for resistance to common root rot. Lines included in the various experiments were current advanced material, registered commercial varieties or deregistered standard varieties. In wheat, the lines screened totalled 64 varieties, 53 breeders' lines from Roseworthy Agricultural College, 95 breeder's lines from the Waite Agricultural Research Institute, and 20 lines from other breeding programs (Table 6.3). In the barley experiments, there were 14 released varieties, including all current commercial varieties, 39 breeders' lines from the Waite Agricultural Research Institute, and six lines from other programs. Each experiment included only a subset of the total lines screened, so the number of tests of each line varied.

There were eight experiments with wheat and five with barley, but some of these were repeated at several sites. Thus, there were totals of 20 wheat and 14 barley locations. Experiment details and site locations are shown in Tables 6.1 and 6.2.

Most experiments were designed expressly for this study, with the two 1984 experiments being performed by Dr A.J. Rathjen (Waite Agricultural Research Institute wheat breeding program) as pilot experiments for this study. Others were breeders' experiments which were sampled opportunistically, because of their having high levels of common root rot, by kind permission of Dr Rathjen, Dr. D.H.B. Sparrow, Mr. G. Hollamby or the Agronomy Unit of the SA Department of Agriculture.

Most experiments were sown, managed and harvested by the wheat or barley breeding programs of the Waite Agricultural Research Institute and Roseworthy Agricultural College (Chapter 3). Two experiments (SWVT/1988 at Tepko and Nangari) were sown by the Agronomy Unit of the SA Department of Agriculture, and differed primarily from the other experiments in plot size and layout (plots 8 rows x 10 m; layout 4 plots lengthwise per bay).

6.2.2. Disease scoring

Severity of common root rot was scored on mature plants (Tables 6.1 and 6.2) (Chapter 3). Generally, samples were brought to the laboratory for scoring on the 0 to 3 scale. This was very time-consuming and in an effort to increase efficiency, two other methods were used in some cases. Firstly, in WV&S/1988 and W800/1988, samples were scored in the field; plants were scored individually and running tallies were kept of the severity score and the total number of plants. The second approach, used on SWVT/1988 and BV&S/1988, was to estimate severity on about 50 plants after quickly examining about 20 of them singly. The proportion of subcrown internode discoloured in the whole sample was estimated, using the same intervals as the standard 0 to 3 scale.

6.2.3. Data analysis

Experiments were analysed by ANOVA to detect significant differences among genotypes in common root rot severity. In experiments with site as a factor, genotype*site interactions were tested. When interactions were significant, new ANOVA's were conducted with each site excluded, one at a time, to detect which site, or sites, gave rise to the significant interaction term. Genotype means of the sites of an experiment not shown to be dissimilar were pooled across the sites. Genotype means from an experiment or site were standardised by expressing them as a percentage of the experiment mean. Within experiments or sites, Tukey's HSD statistics ($p < 0.05$) were calculated for mean comparisons.

Sites and experiments were compared on a pairwise basis, by correlation of genotype severity ratings. In experiments conducted at several sites with no significant genotype*site interaction, pairwise comparisons were also made between the pooled, across-experiment genotype means and those of other sites and experiments. WGxE sites were compared only within the group, as this experiment had too few varieties and degrees of freedom to allow a useful correlation analysis with other sites.

Genotype means were then calculated across all experiments and sites, excluding data from sites which caused significant genotype*site interactions. These means were used to classify wheat genotypes descriptively. They were ordered by size, and plotted in a bar chart, then classified as *moderately resistant* (MR), *moderately susceptible* (MS) or *susceptible* (S). These classifications are as used in the Australian National Rust Control program, in which they are intended to relate to likelihood of economic damage (A.J. Rathjen, personal communication), that is, MR indicates slight losses only occur under severe attack, MS indicates slight losses occur routinely, and S indicates heavy losses occur routinely. Classification of genotypes was done by subjective evaluation of the likelihood of yield loss, based on the proportion of experiments in which a genotype was severely diseased. The assumption that common root rot severity ratings were indicative of yield losses was

justified by the studies of Tinline and Ledingham (1979), as discussed in Section 2.9.4. Barley genotypes were not classified on this basis, since the assumption that disease rating of a genotype indicates the risk of yield loss is less well founded (Tinline and Ledingham, 1979).

Table 6.1. Location and design details of field experiments scored for genotypic resistance in wheat lines to common root rot.

Experiment/year Location	No. of geno-types	No. of repli-cates	Sow-ing date	Samp-ling dates	No. of plants sampled per plot	Mean rainfall (mm)§	Soil type†
W185/1984	39	6					
Sedan			1/6	30/10	10	300	Gc1.2
W581/1985	40	5					
Mannum			13/6	29/10	10	350	Gc1.2
Sedan			20/6	29/10	20	300	Gc1.2
Towitta			12/6	11/11	10	350	Gc1.2
W681/1986	41	5					
Mannum			18/6	19/11	10	350	Gc1.2
Sanderston			17/6	13/11	10	375	Gc1.2
Balaklava			24/6	29/10	10	400	Dr2.23
W781/1987	18	5					
Mannum			26/5	9/11	15	350	Gc1.2
Sanderston Airstrip			29/5	11/11	15	375	Gc1.2
Balaklava 110			2/6	13/11	15	400	Dr2.23
WGxE/1988	9	9					
Mannum			24/5	9/11	15	350	Gc1.2
Sanderston Royal's			27/5	10/11	15	375	Gc1.2
Sanderston Airstrip			25/5	11/11	15	375	Gc1.2
Sanderston Driveway			25/5	11/11	15	375	Gc1.2
Balaklava 110			2/6	13/11	15	400	Dr2.23
Balaklava 170			2/6	13/11	15	400	Dr2.23
WV&S/1988	88	3					
« Mudamuckla			20/5	30/10	25-50	310	Gc1.2
W800/1988	130	3					
« Mudamuckla			19/5	30/10	25-50	310	Gc1.2
SWVT/1988	40	4					
¥ Tepko			24/5	15/11	25-50	375	Gc1.2
Nangari			19/5	17/11	25-50	275	Gc1.2

§ Estimated using nearest station of Bureau of Meteorology
† Codes from Northcote *et al.* (1975).
¥ scored by visual estimate of severity on all plants in sample
« scored for severity only

Table 6.2. Location and design details of field experiments scored for genotypic resistance in barley lines to common root rot.

Experiment/year Location	No. of geno-types	No. of repli-cates	Sow-ing date	Samp-ling dates	No. of plants sampled per plot	Mean rainfall (mm)§	Soil type†
B186/1984	10	6					
« Sedan			1/6	nr	10	300	Gc1.2
B581/1985	10	10					
Mannum			13/6	29/10	10	350	Gc1.2
Sedan			20/6	29/10	20	300	Gc1.2
Towitta			12/6	11/11	10	350	Gc1.2
B681/1986	18	5					
Mannum			18/6	19/11	10	350	Gc1.2
Sanderston			17/6	13/11	10	375	Gc1.2
Balaklava			24/6	29/10	10	400	Dr2.23
B781/1987	18	5					
Mannum			24/5	9/11	15	350	Gc1.2
Sanderston Royal's			27/5	10/11	15	375	Gc1.2
Sanderston Airstrip			25/5	11/11	15	375	Gc1.2
Sanderston Driveway			25/5	11/11	15	375	Gc1.2
Balaklava 110			2/6	13/11	15	400	Dr2.23
Balaklava 170			2/6	13/11	15	400	Dr2.23
BV&S/1988	54	5					
¥ Geranium			24/5	8/11	25-50	390	Gc1.1

§ Estimated using nearest station of Bureau of Meteorology
† Codes from Northcote *et al.* (1975).
¥ scored by visual estimate of severity on all plants in sample
« scored for incidence only

6.3. Results

6.3.1. Wheat

Between the wheat experiments and sites, statistically significant positive correlations ($p < 0.1$) occurred in 43 of the 105 comparisons made (Table 6.4). Six of these involved comparison of a site with the mean of itself and other sites, so that the correlation was artificially improved and must be regarded carefully. On the basis of the other significant correlations, a high degree of repeatability of genotypic response to common root rot was indicated.

In general, strong correlations were associated with multi-site experiment means (W581 and W681), although some individual sites (W185, W581, WV581T,

WV&S88 and W800) showed strong correlations with other sites. Some sites were poorly correlated with all others (W581Se, W681S and SWVT). Some negative correlations occurred, one of which was significant, and this can be attributed to chance due to its low frequency.

Statistical considerations seemed to be the main factor governing the degree to which a site was correlated with others. The more subjective scoring in SWVT made it a poor site, whereas for the W781 sites, there were few degrees of freedom. W581/Towitta and W681/Mannum both had moderate disease severity levels and low error variances, and were frequently highly correlated with other sites. Site-specific factors (e.g. soil type, climate, soil microflora) were probably not important, since W581/Sedan and W781/Mannum, which were poorly correlated with other locations, respectively were adjacent to and in the same paddock as W185 and W681/Mannum, and these were both typical sites.

W681/Sanderston and W781/Sanderston Airstrip were in adjacent paddocks, and both were atypical sites. This casts further doubt on the genotype*site interactions observed, since an interaction due to biotypes might be expected to occur in adjacent paddocks with similar cropping regimes. Neither of the examples here exhibited significant interactions, despite being poorly correlated with other sites. Balaklava 110 was involved in both of the interactions, but the explanation of a spacing effect seems much more likely than a biological cause, given the relatively low severity ratings at that site.

Significant genotype*site interactions were detected in experiments W781/1987 and WGxE/1988 ($p < 0.05$) (Tables 6.4 and 6.5). In both cases, removal of Balaklava 110, and not other sites, changed the interaction term to non-significant. If the interactions were due to different physiological races of *B. sorokiniana*, or to another pathogen such as *F. culmorum*, it is likely that disease reactions would have been correlated between the two sites, and not correlated with others. There were insufficient genotypes in common between the sites to allow their correlation to be

tested, but examination of the data (Table 6.3) showed this not to be the case; in WGxE, reactions at Balaklava 110 of all varieties except one were consistent with ratings in other trials (Table 6.3), while the pooled ratings (from the other sites of WGxE) of some other varieties tended to be low compared to other trials. In W781, several lines at Balaklava 110 were inconsistent with other sites, although others fitted, and, while the site was generally not well correlated with other sites, it was highly correlated with two others, which in turn were not atypical in their responses (Table 6.4). Most correlations in WGxE were weak due to the low degrees of freedom (Table 6.5).

Interactions could conceivably arise through a scaling effect, when the range of values was greater at one site than at the others in the comparisons, so that the regressions differ in slope and appear to intersect. In both experiments, the Balaklava 110 site had low mean severity (Table 6.3) and a limited range in comparison to the other sites, which would support this hypothesis.

There were significant differences among genotypes of wheat in all experiments ($p < 0.001$), which, in general, were quite consistent across sites in spite of the high variability in each experiment (Table 6.3). Ratings of moderately resistant, moderately susceptible and susceptible were divided at 75% and 125% of site means (Figure 6.1). The most resistant had about 25% of the site mean severity of the most susceptible. About 60% of the lines tested were outside the range 75 to 125% of site means. Lines with high levels of resistance included several varieties currently registered in South Australia (e.g. Aroona, Bindawarra, Tatiara and Yarralinka), although other current varieties were among the more susceptible genotypes (e.g. Condor, Excalibur and Machete) (Table 6.3).

The range among breeders' lines was similar to that of registered commercial varieties (Table 6.3), as might be expected, since many have the same genetic background, and specific selection for common root rot is not being practiced. Compared to released varieties, advanced lines in the Waite program seemed to be more resistant

(Experiment W800) (Waite 93.9% of site means vs. varieties 108.5%), while those in the Roseworthy program seemed more susceptible (Experiment WV&S) (Roseworthy 105.1% of site means vs. varieties 96.4%). Neither of the differences were significant by t-test. The hypothesis of difference in the case of Waite lines was based on the premise that unwitting selection for resistance had occurred in yield selection in the F_2 progeny breeding method. Roseworthy also employs this method, but the program is dominated by multiple-backcross derivatives of RAC177, which tend to be highly susceptible to common root rot (asterisked varieties and lines, Table 6.3). Both of the hypotheses were complicated by the presence of recent releases from those programs among the released varieties.

Individual lines from within families tended to be similar in reaction. For example, among 18 lines in the RAC177 family, 14 had mean disease ratings above 100% of site means and only two were classified as moderately resistant (mean 124 ± standard deviation 41%). RAC177 contributes high yielding ability, but clearly lacks resistance to common root rot, which the two exceptional lines (RAC659 and RAC660) appear to have obtained from the donor parents. Both are complex crosses, and it is not justified to surmise a source of the apparent resistance. (MKR*MM) lines, which are closely related to the moderately resistant varieties Bindawarra and Tatiara and are derivatives of Raven (moderately resistant), tended to be rated below experiment means. Two sister F_6 lines from the (WR*Hz)*(GJ*Wq) cross were very different in reaction (susceptible and moderately resistant), indicating that the cross may be segregating, and this is supported by the parental lines (WR*Hz) and(GJ*Wq) appearing to be susceptible and moderately resistant respectively. Two lines of the (MKR*MM) family may also have lost resistance. Among eight lines of the (C8*MM) family, the mean rating was 71%, four lines were rated moderately resistant, and only one, rated at 105%, was above the mean rating of all tests (100%).

6.3.2. Barley

Some barley experiments were highly correlated with each other in disease severity ratings (Table 6.7), but in general, inter-experiment correlation was weaker than in wheat. This was attributable to the high error variances (as indicated by Tukey's HSD statistics) and narrow range (Table 6.6), and the low degrees of freedom in most experiments (Table 6.7).

A significant genotype*environment interaction occurred among the sites of Experiment B581 ($p < 0.001$), but it is difficult to draw any conclusion from this. The high variability within sites made it impossible to attribute the interaction to any particular variety or varieties. As discussed in respect of wheat, an interaction could conceivably arise when the range of values was greater at one site than at others in a comparison, so that the regressions differ in slope and appear to intersect. As was the case for aberrant sites among the wheat experiments, B581/Mannum also had very low mean disease severity. None of the three sites were significantly correlated with each other (Table 6.7), and B581/Mannum was correlated with no other sites, although B581/Sedan and B581/Towitta were significantly correlated with two other sites each at $p < 0.05$.

Within each experiment, there were significant genotype differences (B186, B581, B781 and BV&S, $p < 0.001$; B681, $p < 0.05$). However, there was little discrimination between genotypes, with about 80% of genotypes being within the range 75 to 125% of site means (Figure 6.2). Of the small number of genotypes which seemed appreciably less susceptible than the bulk, just one was a released variety, Waranga, and all were tested once only, making their ratings unreliable.

Table 6.3. Reactions of wheat varieties and breeders' lines to common root rot in field experiments (described in Table 6.1). Genotype severity ratings (0-3) are expressed as percentages of experiment mean severity. Across-site means (¥) do not include reactions at GxE.Balaklava 110 (B110) and W781.B110, due to genotype*site interactions (see Section 6.3). Resistance ratings are *moderately resistant* (MR), *moderately susceptible* (MS) and *susceptible* (S). These were derived from the across-site means (see Section 6.2). Mean separation by Tukey's HSD. Genotypes marked with asterisks (*)at left margin are derivatives of RAC177 (see Section 6.3.1).

	EXPERIMENT											
	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
Site mean severity (0-3)	*0.80*	*0.91*	*0.78*	*1.10*	*1.56*	*0.84*	*1.69*	*2.22*	*2.20*	*2.62*		
Tukey's HSD (p = 0.05) (%)	*123*	*70*	*81*	*101*	*32*	*71*	*18*	*140*	*126*	*110*		
NAMED VARIETIES												
ALDIRK	120	107	112								*113*	*MS*
AROONA	40	86	62	67	96			113	53	48	*71*	*MR*
AVOCET	111										*111*	*MS*
BANKS	88	101									*95*	*MS*
BAYONET	106	107	83	113	116						*103*	*MS*
BINDAWARRA	72	61	69			117	85	60			*71*	*MR*
BLADE		83	87	96	86				53		*78*	*MS*
BODALLIN	67										*67*	*MR*
COCAMBA										105	*105*	*MS*
CONDOR	115	128	105	88	112				114		*114*	*MS*
COOK	108	100									*104*	*MS*
CRANBROOK		107	151								*129*	*S*
DAGGER	73	83	76			118	104		98	96	*89*	*MS*
DARKAN	50										*50*	*MR*
DIAZ									182		*182*	*S*
DOLLARBIRD									167		*167*	*S*
EGRET	170	120	138								*143*	*S*
* EXCALIBUR						120	99	150	144	86	*120*	*MS*
FESTIGUAY	83	93	115					97	45		*87*	*MS*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
GABO	170	122	105								*132*	*S*
GREBE									106		*106*	*MS*
HALBERD	63	86	125	128	104				106	124	*102*	*MS*
KALKEE	82										*82*	*MS*
KAMILAROI (*T. durum*)										96	*96*	*MS*
KATYIL	146	131	138								*138*	*S*
KELELAC									121		*121*	*MS*
KIATA									121	105	*113*	*MS*
KING	132										*132*	*S*
KITE	56	83	82	76	87			90	45	96	*77*	*MS*
KULIN									45		*45*	*MR*
LANCE	82	100									*91*	*MS*
LOWAN									53		*53*	*MR*
* MACHETE	201	123	146	133	108	118	112	165	167	119	*142*	*S*
MADDEN	97	122	102								*107*	*MS*
MATONG									121		*121*	*MS*
MEERING		118	108						91	115	*108*	*MS*
METEOR										115	*115*	*MS*
MILING	163	137	138	128	100						*134*	*S*
MILLEWA	131	82	67			147	89				*94*	*MS*
MINTO									151	105	*128*	*S*
MOKOAN									76		*76*	*MS*
MOLINEUX	88	80	91					158	114	134	*111*	*MS*
MORAY									53		*53*	*MR*
OLYMPIC	79	119	87								*95*	*MS*
OSPREY									114		*114*	*MS*
OXLEY	92	106	105					135	83	153	*112*	*MS*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
RAVEN	71	90	64								*75*	*MR*
ROSELLA									76	57	*67*	*MR*
SABRE	114	94	75								*94*	*MS*
SCHOMBURGK		76	90			102	104	82	30	153	*89*	*MS*
SKUA									45		*45*	*MR*
SONGLEN	103										*103*	*MS*
SPEAR	89	80	84	67	94			90	121	153	*101*	*MS*
SUNCO									144		*144*	*S*
SUNFIELD									106	96	*101*	*MS*
SUNSTAR		112	127								*120*	*MS*
TAKARI		98	114								*106*	*MS*
TATIARA								90	61	48	*66*	*MR*
VASCO									114	86	*100*	*MS*
VULCAN		89							76	124	*96*	*MS*
WARIGAL	90	113	87			129	116	120	83	96	*101*	*MS*
WARIMBA	53	111	100								*88*	*MS*
YALLAROI (*T. durum*)										134	*134*	*S*
YARRALINKA								60			*60*	*MR*
BREEDERS' LINES												
ROSEWORTHY AGRICULTURAL COLLEGE												
* RAC177			154			134	107		98		*121*	*MS*
RAC416		127									*127*	*S*
RAC429	61	79									*70*	*MR*
RAC430			99								*99*	*MS*
RAC495		70									*70*	*MR*
RAC520	173		139			111	129				*145*	*S*

Table 6.3. *continued.*

		W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
	RAC549			144			151	112				*131*	*S*
	RAC557									167		*167*	*S*
	RAC569									151		*151*	*S*
*	RAC580									182		*182*	*S*
	RAC585									144		*144*	*S*
	RAC587									151		*151*	*S*
*	RAC589								90	76	124	*97*	*MS*
	RAC592									121		*121*	*MS*
	RAC596									68		*68*	*MR*
*	RAC597								90	121	134	*115*	*MS*
*	RAC605						105	111	120	151	86	*117*	*MS*
	RAC607								120	106	96	*107*	*MS*
	RAC613								120	121	76	*106*	*MS*
	RAC617									68		*68*	*MR*
	RAC618								105	91	105	*100*	*MS*
*	RAC620									182		*182*	*S*
*	RAC622									159		*159*	*S*
	RAC623									114		*114*	*MS*
	RAC627									76		*76*	*MS*
	RAC628								105	106	115	*109*	*MS*
*	RAC629								60	106	105	*90*	*MS*
	RAC633								105	151	67	*108*	*MS*
*	RAC635									167		*167*	*S*
	RAC636									129		*129*	*S*
	RAC637									76		*76*	*MS*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
RAC638									106		*106*	*MS*
RAC639									30		*30*	*MR*
RAC640									45		*45*	*MR*
RAC641									83		*83*	*MS*
RAC643									60		*60*	*MR*
RAC644									91		*91*	*MS*
RAC645									76		*76*	*MS*
* RAC646									182		*182*	*S*
* RAC647									91		*91*	*MS*
* RAC648									136		*136*	*S*
* RAC649									121		*121*	*MS*
* RAC650									114		*114*	*MS*
RAC651									68		*68*	*MR*
RAC652									76		*76*	*MS*
RAC653									61		*61*	*MR*
RAC654									106		*106*	*MS*
RAC655									61		*61*	*MR*
RAC656									76		*76*	*MS*
RAC657									114		*114*	*MS*
RAC658									76		*76*	*MS*
* RAC659									53		*53*	*MR*
* RAC660									30		*30*	*MR*
BREEDER'S LINES												
WAITE AGRICULTURAL RESEARCH INSTITUTE												
(C-3*MKR**3)*MKR/1/10)/11/3								90			*90*	*MS*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
(C-3*MKR**3)*MKR/1/10)/11/8								105			*105*	*MS*
(C8*MM/17)/10/7			76								*76*	*MS*
(C8*MM)*(MM*MMC)/45/8						51	80				*73*	*MR*
(C8*MM)*(MM*MMC/47/15)/45/3								90	61	48	*66*	*MR*
(C8*MM)*(MM*MMC/47/15)/45/7								30			*30*	*MR*
(C8*MM)*(MMC*Wm)/13/1								105			*105*	*MS*
(C8*MM)*MKRK/7/14)/18/13								90			*90*	*MS*
(C8*MM)*MKRK/7/14)/18/9								45			*45*	*MR*
(C8*HR)*MM)/W31/S20	83										*83*	*MS*
(CH8*Wb)*(MM*MMC/29/8)/13/19								60			*60*	*MR*
(CH8*Wb)*(MM*MMC/29/8)/8/11								60			*60*	*MR*
(CH8*Wb)*(MM*MMC/29/8)/8/8								120			*120*	*MS*
(ExBelg2*Aroona)/8/20								105			*105*	*MS*
(Fund*CP)(MMC*Wm)/2/9								135			*135*	*S*
(Fund*CP)*WL-C3)*Wl)/22/15								60			*60*	*MR*
(GJ*WQ)*MKRK/13/11)/21/1								120			*120*	*MS*
(GJ*WQ)*MKRK/7/14)/7/3								38		67	*52*	*MR*
(GP*By)*PF/41/1)/7/14								120			*120*	*MS*
(GP*Ox)*(Wl*MMC/7/13)/1/15								90			*90*	*MS*
(MKR*By)*(GJ*Wq)/10/24								60			*60*	*MR*
(MKR*By)*(GJ*Wq)/10/30								90			*90*	*MS*
(MKR*MM)*(GJ*Wq)/1/10								90			*90*	*MS*
(MKR*MM)*(GJ*Wq)/7/7								180			*180*	*S*
(MKR*MM)*(Wl*MMC)/5/1								120			*120*	*MS*
(MKR*MM)*(Wl*MMC)/5/7								90			*90*	*MS*
(Tatiara) (MKR*MM)/W18/18			59								*59*	*MR*
(MKR*MM/18/18)*(C8H*MM)/4/1								75			*75*	*MR*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
(MKR*MM/18/18)*(C8H*MM)/4/4						57	87	60			*72*	*MR*
(MKR*MM/18/18)*MKRK/13/11)/1/14								90			*90*	*MS*
(MM*MMC)*WI-ar)/13/13								90			*90*	*MS*
(MMC*MM)*WI-ar)/106/6/3								75			*75*	*MR*
(MMC*Wq)*(GJ*Wq)/7/5								105			*105*	*MS*
(MMC*Wq)*(GJ*Wq)/9/3								60			*60*	*MR*
(MMC*Wq)*CP)*(MM*MMC)/2/12								165			*165*	*S*
(MMC*Wq)*CP)*MKRK/13/8)/13/13								75			*75*	*MR*
(MMC*Wq)*MKRK/13/11)/7/26								105			*105*	*MS*
(MMC*Wq)*MKRK/13/8)/6/3								60			*60*	*MR*
(MKR*K)/S13/S8	134										*134*	*S*
(MKR*K)/W35/3	44	99									*71*	*MR*
(Tatiara) (MKR*MM)/W18/18						57	87				*80*	*MS*
(Naofen*WmH/30/2)/8/14								60			*60*	*MR*
(O8*MKR)*(GJ*Wq)/12/3								105			*105*	*MS*
(O8*MKR)*(GJ*Wq)/12/4								120			*120*	*MS*
(SKR*MKR*3)*MKR/9/17						68	88				*83*	*MS*
(TJB*MKR)*MKRK/13/11)/13/11								90			*90*	*MS*
(TJB*MKR)*MKRK/13/8)/12/25								75			*75*	*MR*
(TJB*MKR)*MKRK/13/8)/12/30								90			*90*	*MS*
(TJB*MKR)*MKR/13/8)/12/4								60			*60*	*MR*
(TJB*MKR)*WI-ar)/9/12								90			*90*	*MS*
(TT2*W1*3)*Aroona)/14/20						66	79				*76*	*MS*
(Vd*MM**2)/29/W6						54	122				*105*	*MS*
(Vd*Wk**2)*WmH/30/2)/3/3								75			*75*	*MR*
(Vd*Wq**2)*PF/41/4)/16/11/5								120			*120*	*MS*
(Vil*(MMC*Wq)/14/18								60			*60*	*MR*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
(WI-TR/15/3*(WI*MMC/7/13)/1/14								75			*75*	*MR*
(WI-TR/22/4*(MM*MMC/57/17)/10/10								150			*150*	*S*
(WI-TR/33/1*(WI*MMC/7/13)/15/14								90			*90*	*MS*
(WI-TR/33/1*(WI*MMC/7/13)/15/25								45			*45*	*MR*
(WMH/36/3*WL-C3)/11/6								90			*90*	*MS*
(Wq*08)*WI)/4/2								60			*60*	*MR*
(Wq*Hz)*PF/41/1)/20/2								105			*105*	*MS*
(WQ*KP)*Bay)/20/5			98			68	105	105			*101*	*MS*
(WQ*KP)*WMH)/6/12			54			84	69	90			*72*	*MR*
(WR*Hz)*(GJ*W9)/15/33								90		67	*79*	*MS*
(WR*Hz)*(GJ*Wq)/15/21								135			*135*	*S*
(WR*Hz)*(GJ*Wq)/15/23								45			*45*	*MR*
(WR*Hz)*(MM*MMC)/32/12								105			*105*	*MS*
(WR*Hz)*(MM*MMC)/32/13								105			*105*	*MS*
(WR*Hz)*(MM*MMC)/32/18								90			*90*	*MS*
(WR*Hz)*(WI*MMC)/2/9								113			*113*	*MS*
(WR*Hz)*MKRK/13/8)/11/23								105		105	*105*	*MS*
(WR*Hz)*MKRK/13/8)/11/3								105			*105*	*MS*
(WR*Hz)*MKRK/13/8)/11/4								113		96	*104*	*MS*
(WR*Hz)*MKRK/13/8)/20/1								90			*90*	*MS*
(WR*Hz)*MKRK/13/8)/4/2								120			*120*	*MS*
(WR*Hz)*PF/41/1)/3/23								90			*90*	*MS*
(WR*MM)*(MM*MMC)/6/20								105			*105*	*MS*
EP(Aroona*(Joss*MMC)/18/16								75			*75*	*MR*
EP(Aroona*(Joss*MMC)/18/5								120			*120*	*MS*
EP(Aroona*(Joss*MMC)/21/7								135			*135*	*S*
EP(Aroona*(Joss*MMC)/4/9								105			*105*	*MS*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
EP(Aroona*(Mois*Wb)/10/6								105			*105*	*MS*
EP(Aroona*Insignia)/3/15								45			*45*	*MR*
EP(Bodallin*Halberd)/10/1								105			*105*	*MS*
EP(Cook*Spear)/23								90			*90*	*MS*
EP(Millewa*(Ar+Sr27)/9/3								135			*135*	*S*
EP(Millewa*(Mois*Wb)/15/8								150			*150*	*S*
EP(MMG*MKR)*Spear)/31								105			*105*	*MS*
EX(C8H*MM)*NJ28)/20								135			*135*	*S*
(Vd*MM**2)/29/W2		78	84								*81*	*MS*
PF/41/W1			92								*92*	*MS*
Yr10Schomburgk										105	*105*	*MS*
Yr10Warigal										76	*76*	*MS*
Aroona PT 7/5		82									*82*	*MS*
BREEDERS' LINES												
OTHER PROGRAMS												
AD-11AG/4*CON/3/TM53/WW15/SE18*2								90			*90*	*MS*
AD-8VIC001/KALKEE/AUSEN111-46/3/AG*4C								90			*90*	*MS*
BD062								150			*150*	*S*
BD116								105			*105*	*MS*
BD159								120			*120*	*MS*
BL001								120			*120*	*MS*
BL009								165			*165*	*S*
BL023								120			*120*	*MS*
BL044								150			*150*	*S*
IW562									61		*61*	*MR*
IW610									129		*129*	*S*

Table 6.3. *continued.*

	W185	W581 mean	W681 mean	GxE B110	GxE Other sites	W781 B110	W781 Other sites	W800	W V&S	SWVT	*Across -site means ¥*	*Rating*
K1115									83		*83*	*MS*
QT3344									98		*98*	*MS*
WW731									61		*61*	*MR*
XD85										86	*86*	*MS*
YD-14(ND50*Condor)*DX-6-79RR)								135			*135*	*S*
ZD-11Oxley*DX6-148								150			*150*	*S*
ZD-24(ND136/Egret)*Cook								135			*135*	*S*
SUN110C									91		*91*	*MS*
CANADIAN 1008-C16			69								*69*	*MR*

Table 6.4. Correlations of genotype ratings of common root rot severity between experiments on wheat. Values below diagonal are correlation co-efficients. Asterisks indicate statistical significance: * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$. Values above diagonal are the number of genotypes occurring in both experiments and contributing to the estimate. Experiments with multiple sites were pooled into means if the site*genotype interaction was not significant. Site name abbreviations are M=Mannum, Se=Sedan, T=Towitta, S=Sanderston, B=Balaklava, A=Airstrip (see Table 6.1)

correlation coefficients	W185	W581 mean	W581 M	W581 Se	W581 T	W681 mean	W681 M	W681 S	W681 B	W781 mean	W781 M	W781 SA	W781 B110	WV&S8 8	W800	SWVT
W185		27	27	27	27	20	20	20	20	not	6	6	6	12	10	8
W581 mean	0.59***		39	39	39	30	30	30	30	tested	9	9	9	15	10	12
W581M	0.35*	0.80***		39	39	30	30	30	30		9	9	9	15	10	12
W581Se	0.16	0.46***	0.11		39	30	30	30	30		9	9	9	15	10	12
W581T	0.64***	0.74***	0.42**	-0.30		30	30	30	30		9	9	9	15	10	12
W681 mean	0.69***	0.65***	0.44**	0.29	0.60***		39	39	39		8	8	8	14	12	11
W681M	0.69***	0.63***	0.41**	0.16	0.69***	0.79***		39	39		8	8	8	14	12	11
W681S	0.41*	0.32*	0.28	0.33*	0.09	0.76***	0.28		39		8	8	8	14	12	11
W681B	0.42*	0.39**	0.29	0.08	0.40**	0.74***	0.34	0.57***			8	8	8	14	12	11
W781 mean			significant genotype*site interaction, so not tested													
W781M	-0.13	0.18	0.15	-0.03	0.27	0.50	0.44	0.28	0.49			18	18	6	8	6
W781SA	0.50	0.62*	0.45	0.31	0.72**	0.16	0.48	-0.13	0.34		0.38		18	6	8	6
W781B110	0.52	0.65*	0.38	0.41	0.80***	0.44	0.60	0.22	0		0.28	0.74***		6	8	6
WV&S88	0.84***	0.48*	0.35	0.12	0.49*	0.56**	0.32	0.40	0.57**		0.22	-0.67	-0.80*		20	29
W800	0.64*	0.74**	0.51	0.41	0.68**	0.58**	0.76***	0.04	0.54*		-0.40	0.39	0.09	0.51**		24
SWVT	0.37	0.10	-0.06	-0.20	0.31	0.06	0	0.14	0		-0.73**	0.35	0.21	0.41**	0.18	

	Location				
	Balaklava 110	Mannum	Sanderston Airstrip	Balaklava 170	Sanderston Royal's
Mannum	0.49				
Sanderston Airstrip	0.34	0.27			
Balaklava 170	0.40	0.43	0.55		
Sanderston Royal's	0.58*	0.84***	0.63*	0.46	
Sanderston Driveway	0.21	0.51	0.63*	0.93***	0.59*

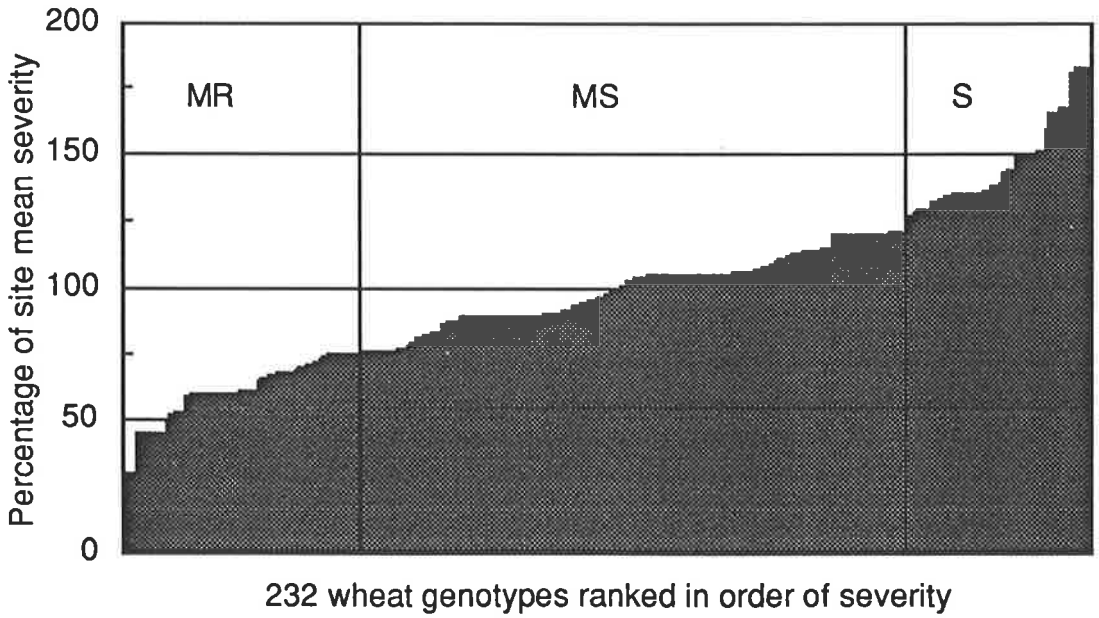


Table 6.6. Reactions of barley varieties and breeders' lines to common root rot in field experiments. Ratings are expressed as percentages of site mean severity. Experiment names are explained in Table 6.2. Mean separation by Tukey's HSD.

	EXPERIMENT							
	B186	B581 Man-num	B581 Sed-an	B581 Tow-itta	B681 mean	B781 mean	B V&S	*mean*
Site means (0-3 scale)	*44.6*	*0.73*	*1.62*	*1.11*	*1.10*	*1.69*	*1.30*	
Tukey's HSD (0.05)%	*20*	*68*	*38*	*68*	*49*	*23*	*111*	
CLIPPER	112	122	89	107	94	110	85	*104*
DAMPIER					117	116		*117*
FORREST	157	71	127	154	124	114	92	*120*
GALLEON	68	76	95	64	92	95	123	*91*
GRIMMETT		81	79	103	85	92		*88*
MOONDYNE							92	*92*
O'CONNOR		140	120	126	100	96	116	*104*
PARWAN		88	116	97	88	108		*100*
SCHOONER	95	96	88	106	102	95	108	*98*
SHANNON		107	67	81	88	95		*90*
SKIFF		91	108	93	85	102	92	*95*
STIRLING	119	128	111	70	101	93	100	*99*
WARANGA							77	*77*
WEEAH	156				113	111	92	*115*
WI-2505							85	*85*
WI-2585							169	*169*
WI-2597							116	*116*
WI2645	95				84	95	92	*90*
WI2646	145				117	109	169	*121*
WI-2666							62	*62*
WI2668					107	103	92	*102*
WI2669					99	92		*94*
WI2674					86	95	131	*94*
WI-2675							108	*108*
WI-2685							100	*100*
WI-2687							62	*62*
WI-2688							108	*108*
WI-2689							54	*54*
WI-2690							108	*108*
WI2692					118	80	92	*93*
WI-2693							69	*69*
WI-2722							92	*92*
WI-2723							108	*108*
WI-2727							131	*131*
WI-2728							92	*92*
WI-2729							108	*108*
WI-2733							92	*92*
WI-2734							92	*92*
WI-2736							92	*92*
WI-2737							169	*169*

Table 6.6. *continued.*

	B186	B581 Man-num	B581 Sed-an	B581 Tow-itta	B681 mean	B781 mean	B V&S	*mean*
WI-2738							108	*108*
WI-2803							85	*85*
WI-2804							108	*108*
(((MIDAS*AKKA)/3*CLIP)*WI-2472)/33							77	*77*
((2105*(CPI*CAMB)/4*(CLIP*AKKA))/25							85	*85*
((2105*(CPI*CAMB)/4*WI-2468)/59							100	*100*
((2105*(CPI*CAMB)/4*WI-2468)/9							100	*100*
((AKKA*WI2231)*WI-2468)/13							139	*139*
((CLIP*CPI)/14*2EBYT23)/17							116	*116*
((CLIPPER*COHO)*WI-2468)/14							46	*46*
((ESTANZ*CLIP)*WI-2468)/16							92	*92*
((O.PROPHETE*WI--2231)*WI-2468)/22							92	*92*
(WI-2105*WI-2472)/1							92	*92*
VIC-24-86011							69	*69*
WA-74S/311							116	*116*
WA-75S/323							92	*92*
WA-75S/329							100	*100*
WA-77S/399							92	*92*
WA-77S/400							116	*116*

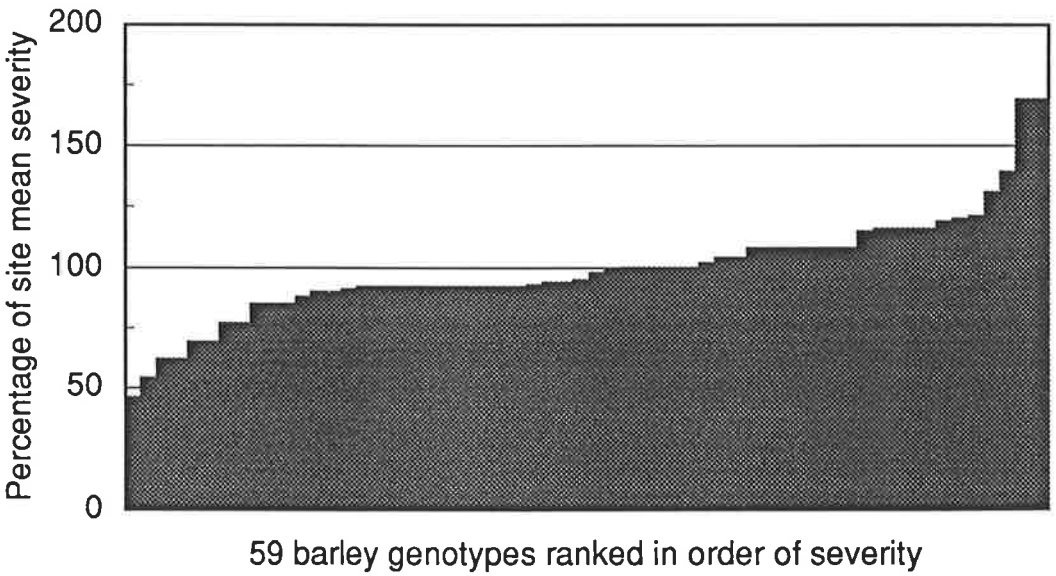


Figure 6.2. Mean percentages of site mean severity of common root rot of 59 barley genotypes (see Table 6.7), ordered from lowest to highest severity from left to right.

Table 6.7. Correlations of genotype ratings of common root rot severity between experiments on barley. Values below diagonal are correlation co-efficients. Asterisks indicate statistical significance: * = $p < 0.1$, ** = $p < 0.05$, *** = $p < 0.01$. Values above diagonal are the number of genotypes occurring in both experiments and contributing to the estimate. Experiments with multiple sites were pooled into means if the site*genotype interaction was not significant. Site name abbreviations are M=Mannum, Se=Sedan, T=Towitta, S=Sanderston, B=Balaklava, A=Airstrip, R=Royal's, D=Driveway (see Table 6.2).

correlation coefficients	B186	B581 M	B581 Se	B581 T	B681 mean	B681 M	B681 S	B681 B	B781 mean	B781 B110	B781 B170	B781M	B781 R	B781 SA	B781 SD	BV&S 88
B186		5	5	5	8	8	8	8	8	8	8	8	8	8	8	8
B581 mean	significant genotype*site interaction, so not tested															
B581M	0.01		10	10	10	10	10	10	10	10	10	10	10	10	10	6
B581Se	0.80	0.03		10	10	10	10	10	10	10	10	10	10	10	10	6
B581T	0.78	-0.08	0.47		10	10	10	10	10	10	10	10	10	10	10	6
B681 mean	0.85 ***	0.06	0.55*	0.65**		18	18	18	18	18	18	18	18	18	18	13
B681M	0.78**	-0.24	0.64**	0.49	0.90***		18	18	18	18	18	18	18	18	18	13
B681S	0.04	0.11	0.03	0.46	0.40	0.06		18	18	18	18	18	18	18	18	13
B681B	0.74**	0.30	0.33	0.74 **	0.82***	0.59***	0.25		18	18	18	18	18	18	18	13
B781 mean	0.79**	-0.23	0.48	0.58*	0.66**	0.55**	0.14	0.66***		18	18	18	18	18	18	13
B781B110	0.32	-0.59*	-0.02	0.27	0.38	0.26	0.19	0.41	0.73***		18	18	18	18	18	13
B781B170	0.77**	-0.33	0.58*	0.59*	0.68**	0.68***	0.07	0.50**	0.90***	0.54**		18	18	18	18	13
B781M	0.69*	-0.10	0.64**	0.60*	0.43*	0.35	0.03	0.48**	0.71***	0.72***	0.52**		18	18	18	13
B781R	-0.06	-0.60*	0.32	0.24	0.20	0.16	0.07	0.20	0.60***	0.38	0.57**	0.31		18	18	13
B781SA	0.63*	0.02	0.23	-0.07	0.40	0.36	0.07	0.42*	0.73***	0.29	0.73***	0.25	0.54**		18	13
B781SD	0.37	-0.02	0.19	0.41	0.36	0.20	0.27	0.43*	0.38	0.20	0.22	0.20	-0.16	0.01		13
BV&S88	0.03	-0.11	-0.05	-0.41	0.09	0.18	-0.31	0.09	-0.09	-0.07	0.04	-0.16	-0.46	-0.38	0.44	

6.4. Discussion

6.4.1. Wheat

In wheat, the results confirmed the preliminary studies of Lewis (personal communication), that there was variability in common root rot resistance among South Australian varieties and breeding lines. The range of resistance was substantial, from lines which showed negligible levels of disease even under high disease pressure, to those which became severely diseased even when site mean disease severity was low (Table 6.3). These results reflect previous reports from North America and Queensland (see Section 2.9.3). Susceptibility indices of 21 varieties were correlated with data of Wildermuth and McNamara (1987b) from one test in Queensland ($r^2 = 0.52$, $p \leq 0.001$).

The potential value of the moderate level of resistance observed was demonstrated in wheat crops surveyed for common root rot (Chapter 4); varieties with mean disease severity of approximately 75% of site means in the resistance studies (Table 6.3), had significantly less severe disease than more susceptible varieties (Tables 4.4 and 4.5). These more resistant varieties comprised only a small proportion (14%) of all crops surveyed. Hence, selection for resistance could substantially reduce the mean severity of common root rot of South Australia's wheat crop. It would be reasonable to adopt the rating of the most resistant variety in the survey, Aroona (71% of site means, Table 6.3), as a goal or standard if breeding was undertaken.

Whether such a change in mean resistance ratings in the state's wheat crop could be achieved depends on the factors listed by Tinline *et al.* (1989), *viz.*:

- good sources of genetic resistance are required;
- the inheritance of resistance in the host must be known;
- virulence in the pathogen must be known; and
- it must be feasible to screen for resistance.

Furthermore, for resistance breeding to be adopted, the disease would not be adequately and efficiently controlled by other methods. Finally, for resources to be

transferred to breeding for resistance to common root rot, evidence is required that an economic benefit would be achieved. These issues are discussed below.

The results indicated that resistance better than Aroona's is common among adapted varieties and advanced lines in the breeding programs (Table 6.3). Fortyone lines were rated equal to or better than Aroona. The most resistant variety among those tested at several sites, Tatiara, had a mean disease severity of 66% of site means. Among genotypes tested more than twice, only one, (C8*MM)(MM*MMC/47/15)/–45/3, had a rating equal to or better than Tatiara's, but among lines tested once or twice, there were 27 which were rated as more resistant, out of 232 lines in total.

Hence, there would be no justification in introducing resistance in unadapted material, such as early maturing or red-grained Canadian varieties, or the germplasm lines released by DePauw *et al.* (1984), or the alien sources screened by Bailey (1987).

Because of the relative consistency of ratings which was observed within families of lines, it can be assumed that the resistance is similar in inheritance to that described previously. That is, it may be polygenic, but with relatively few genes (Sallans and Tinline, 1965; McKenzie and Atkinson, 1968; Larson and Atkinson, 1981; Stack, 1982; Bailey *et al.*, 1988) and quite high heritability (Bailey *et al.*, 1988).

It is important that resistance should be known to be stable, or consistent across the relevant cropping area, before it is adopted as a selection criterion. The significant genotype*site interactions which were observed (Tables 6.3 and 6.4), were probably artefacts of a scaling effect of disease severity between sites (see Section 6.3), rather than attack by physiological races or by other common root rot pathogens; neither of these complex hypotheses are justified, since in the aberrant trials, the ratings of lines were generally consistent with other trials (Tables 6.3 and 6.4). There is no strong evidence in the literature to indicate that *B. sorokiniana* might overcome resistance in wheat to common root rot (see Section 2.9.3). Also, as discussed elsewhere (Chapter 5), there is no convincing evidence that pathogens besides *B. sorokiniana* cause common root rot in South Australia.

The results indicated that it is feasible to screen for resistance to common root rot in South Australia, without substantial change to the breeding programs. Firstly, if common root rot reduces yield in the state, the F_2 progeny method should select for resistance, since screening for yield is done under commercial conditions in the field when maximum variability exists among genotypes (see Section 6.1). Indeed, the high frequency of moderately resistant genotypes among the varieties and breeding lines (Table 6.3) constitutes evidence that unwitting selection has occurred.

Specific selection could be done on the basis of field ratings. While genotypes were rated quite consistently across several sites, ratings from just one or two sites may not be adequate for selection, since aberrant ratings occurred at single sites with varieties which were determined in several tests to be either moderately resistant or susceptible. It would be feasible to either discard lines with high ratings, or select lines with low ratings. It was beyond the scope of this study to determine at what intensity selection should be done to achieve a desired rate of genetic improvement. Further, there are the pragmatic considerations of what is feasible within existing breeding priorities.

Two approaches could be used for screening, either sowing in nurseries (in which rotations were designed to maximise inoculum potential of *B. sorokiniana*), or sowing at several sites and choosing the best for screening. The first option requires long-term investment, and care to avoid problems with cereal cyst nematode (*H. avenae*) and take-all (*G. graminis* var. *tritici*), arising from frequent cereal cropping. The second option, to avoid a large investment, is best done by opportunistic sampling of breeders' trials, but still has a risk of poor results when low disease levels occur.

The field evaluation done for Experiments W800 and WV&S was comparable in accuracy to rating done in the laboratory for the earlier experiments. Variability in these two experiments was relatively high, but this was probably due to the low level of replication rather than any difference in scoring accuracy. Therefore, choice of where to conduct the ratings, in the field or in the laboratory, depends on practical considerations such as proximity of site to station, and facilities for transport and

laboratory work. Plants can also be stored prior to scoring, since in the earlier experiments, this appeared not to result in appreciable deterioration in the clarity of symptoms. The less objective rating system used in SWVT, in which about 20 plants were scored together, rather than individually, was much faster, but inferior in accuracy (Table 6.3). The main difficulty was in discriminating between genotypes under high mean disease severity; there was a tendency to weight heavily severely diseased plants, so that many genotypes were rated as susceptible.

Selection from crosses at an earlier generation may not be feasible in the field, due to both of the problems mentioned above, and the requirement for large numbers of lines to be screened.

It should be possible to devise a satisfactory method for screening plants in the glasshouse. Some studies have found seedling tests to be of little use in North America (Harding, 1971; Stack, 1985), but it is not clear whether the two phases of the disease are covered by different genetic systems. Alternatively, the resistance observed in adults may simply be overwhelmed by the rapid invasion of immature tissue. The conclusion that seedling tests cannot be used may not be valid for the much longer season varieties of Australia (about 170 days growing season, versus about 100 days). Harding's (1971) study seemed to relate to seedling blight rather than common root rot. Stack's (1985) seedling ratings, which showed no differences, were at an early stage of disease progression and, as shown in reports on progression of common root rot (Verma 1982, Stack 1980, Bailey *et al.* 1989), varieties cannot be separated until severity has progressed to moderate levels, later in the season. The longer season of Australian wheats, with manipulation of experimental conditions (see Section 5.2.3), might allow moderate severity to be attained early (say 7 to 10 weeks) without inducing seedling blight.

Statistical considerations seemed to be the main factor in making a site typical or not, in terms of the degree to which it was correlated with other sites. The more subjective scoring in SWVT made it a poor site, whereas for the W781 sites, there were few

degrees of freedom. W581/Towitta and W681/Mannum both had moderate disease severity levels and low error variances, and were frequently correlated with other sites.

Location may not govern the degree of correlation, since W581/Sedan and W781/Mannum, which were less representative than others, respectively were adjacent to and in the same paddock as W185 and W681/Mannum, and these were both typical sites. However, W681/Sanderston and W781/Sanderston Airstrip were in adjacent paddocks, and both were atypical sites. This casts further doubt on the genotype*site interactions observed, since an interaction due to biotypes might be expected to occur in adjacent paddocks with similar cropping regimes. Neither of the examples here exhibited significant interactions, despite being poorly correlated with other sites.

The question of whether other methods of control are adequate and make resistance breeding unnecessary was answered elsewhere in this thesis. Studies on the effects of non-host rotations at one site (Chapter 7) indicated that reductions in disease levels may be achieved, and this was supported by crop survey results, in which there was less common root rot in regions where the climate permits rotation of wheat with sown legume crops and pastures (Chapter 4). However, these alternatives to wheat cannot be grown in drier regions of the state (Taylor, 1991). Also, light soil types, which may aggravate common root rot (Chapter 7), predominate in these regions (Wright, 1969). Fungicidal seed-dressings may be beneficial in the drier regions, if the risk of phytotoxicity is managed by shallow sowing (Chapter 8). Direct drilling may also be beneficial (Chapter 7), although many farmers do not have the required equipment, or cannot, for some other reason, adopt this technology. Hence, resistance to common root rot has a potentially large role in wheat farming in South Australia.

The question remaining is whether yield losses justify changing breeding priorities, in order to accommodate resistance to common root rot. Studies reported by Whittle *et al.* (1991) indicated that substantial yield losses from common root rot occurred

frequently in South Australian wheat crops, although there were ambiguities in the data. When these results are considered with the evidence that the disease is widespread in the state and can be severe, given common seasonal conditions (Chapter 4), it seems likely that yield losses of 5 to 15%, as observed in Canada (see Section 2.6) may occur. Even mean losses at the low end of this range may justify resistance breeding.

6.4.2. Barley

The level of genetic variability observed in wheat was not seen in barley. In general, barley sites and experiments were less well correlated with each other than with wheat (Table 6.7). However, significant correlations still occurred, showing that the differences between genotypes were repeatable, though small, compared to wheat. Since none of the genotypes, which included all current Australian commercial varieties, could be rated definitively as moderately resistant, breeding in this crop would require introduction of material with greater resistance, and so would be much more complex. There are no reports in the literature of of resistance in barley equivalent in effect to that found in wheat, and information on the inheritance of resistance is scarce. The inheritance of resistance has been studied less with respect to barley than to wheat, probably because of the paucity of effective resistance in barley, and the known difficulties with non-genetic variability in studies on this crop (Cohen *et al.*, 1969, Duczek, 1984).

Because of the lack of genetic variability and high error variances in experiments, screening barley in the field is difficult, and improved testing procedures are required. As discussed for wheat above, Stack's (1985) rejection of seedling screening, while applicable for North American barleys, might not apply for long-season Australian genotypes. *In vitro* tests using toxin produced by *B. sorokiniana* might be useful (Stack, 1992), but recent results (Stack, personal communication) indicate that this is not likely.

7. EFFECTS OF ROTATIONS, TILLAGE AND SOIL TYPE ON INOCULUM DENSITY OF *B. SOROKINIANA* IN SOIL

7.1. Introduction

The purpose of this study was to examine the effects of three important aspects of South Australian farming systems, rotations, tillage and soil type, on *B. sorokiniana* inoculum density in soil. This was done over two years in a long-term field trial.

Rotation of crops is an effective method of disease control for some pathogens, the lifecycles of which may be interrupted by the use of one or more resistant crops; in South Australia, particular rotations are recommended for the control of take-all (*G. graminis* var. *tritici*) and cereal cyst nematode (*H. avenae*) in wheat. A range of rotational crops and plant species have been shown elsewhere to influence soil inoculum densities of *B. sorokiniana* (Ledingham, 1961; Chinn, 1976b; Reis and Wünsche, 1984; Kidambi *et al.*, 1985; Sturz and Bernier, 1987; Piening and Orr, 1988; Wildermuth and MacNamara, 1991), but some of the rotations in the long-term field trial reported here have not been studied previously. Butler (1961) was of the opinion that rotations controlled common root rot to a limited degree in Australia, and this was supported by the finding of Wildermuth and MacNamara (1991) that inoculum density lowered by a non-host crop was returned to a high level by a single crop of wheat, but no reports had been published from South Australia, or from similar climatic and edaphic conditions.

The second factor studied was tillage practice. Soil preparation in South Australia usually involves two or more cultivations prior to sowing, to control weeds and to prepare a seedbed. To lower costs, improve soil structure and reduce erosion risk, many farmers are reducing the frequency and intensity of cultivation, and some are "direct drilling", with no preparatory cultivation and minimal ground disturbance at sowing. Herbicides are used for weed control in these systems. Tillage can increase the risk of some diseases, by dispersing the pathogen (e.g. cereal cyst nematode), but it can also reduce disease, by reducing inoculum potential (e.g. Rhizoctonia barepatch

(*Rhizoctonia solani*) of wheat) (Rovira, 1987). Soil inoculum density of *B. sorokiniana* with direct drilling can be half of that with conventional cultivation (Duczek, 1981; Reis and Abrao, 1983), but this effect may be less pronounced with "minimum tillage" (Conner *et al.*, 1987).

The effects of two soil types on inoculum density of *B. sorokiniana* were also studied. Some indications that soil type can affect *B. sorokiniana* inoculum density were found by Duczek (1981) and Wildermuth (1986), but the most conclusive evidence was from Samuel (1924), who in South Australia, found the fungus infecting a high proportion of wheat plants on sandhills, but few in the adjacent flats of sandy loam.

7.2. Materials and methods

7.2.1. Field trial details

The CSIRO long-term trial at Avon was used, by kind permission of Dr. A.D. Rovira. General details of the trial are given here, but a fuller description of the site and the trial design was given by Rovira and Venn (1985).

The experiment was a factorial arrangement of rotations (five), tillage practices (two) and soil types (two), with six replicates. Rotations and tillage practices conformed with a randomised block design, but the soil types were stratified (see below).

(1) the rotations were all sown to wheat in even-numbered years and the "alternate phase" crop or pasture in odd-numbered years, commencing with wheat in 1978. Alternate phases were:

- wheat (after 1986 cv. Spear [moderately susceptible to common root rot (Chapter 6)]);
- grassy pasture (volunteer mixture of barley grass (*Hordeum glaucum*), ryegrass (*Lolium rigidum*), and annual medic (*Medicago littoralis* cv. Harbinger);
- field peas (*Pisum sativum*);
- sown medic pasture (*M. scutellata*); and

- oats (*Avena sativa*).

(2) the tillage treatments were:

- direct drilling (DD) (no pre-sowing tillage, minimum disturbance at sowing using coulters and narrow shares, weeds controlled with a mixture of paraquat (0.125 kg/L) and diquat (0.075 kg/L) (Spray Seed; ICI Australia Ltd.) 2 days before seeding); and
- conventional cultivation (CC) (cultivated to 7 cm three times before sowing with a combine drill with 15 cm shares, and sown with the same implement).

The oats rotation was only included in the CC treatment, as this crop does not thrive when direct-drilled;

(3) the soil types were an alkaline calcareous sandy loam and a calcareous clay loam (classifications Gc1.12 and Gc.2.12 respectively (Northcote *et al.*, 1975)), which are the two major soil types on South Australian cereal farms. The trial site incorporated a sandy loam hillside and the clay loam swale at its base. Plots were 100 m in length and 10 drill rows in width, sown perpendicular to the contour, so that one end (southern) was on the clay loam swale and the other end was on the sandy loam slope.

The study reported here was conducted over two years, covering both phases of the rotations. The first year was a pilot study to determine suitability of sampling methods. That year followed the alternate rotational phases (1987) and the second year followed the wheat phase (1988).

Wheat was sown too shallow for the formation of subcrown internodes, precluding measurement of common root rot severity on the subcrown internode (Chapter 3). Altering sowing depth was not an option, since the trial had other primary objectives including the measurement of grain yields and monitoring of *G. graminis* var. *tritici*

levels. Hence, measurements were limited to assessment of inoculum density of *B. sorokiniana* in the soil.

7.2.2. Soil sampling and plating

Soil samples for the first year, from the alternate phases, were archival samples, not taken for this study. They were taken from the 0 to 5cm horizon in September, 1988, during the growth of the wheat phase, and air-dried in open containers in a glasshouse. Samples from the six replicates were combined.

Soil samples from the wheat phase were taken on 11 April, 1989. This followed the usual dry summer and was prior to the opening rains, so the soil was air dry. Cultivation of the CC treatment had not commenced and the alternate phases had not been sown. Stubble had been heavily grazed by sheep. Samples were taken from the first three replicates of each rotation on the sandy loam and in the wheat/wheat, pasture/wheat and medic/wheat rotations on the clay loam. The peas/wheat and oats/wheat rotations in the clay loam were omitted due to high levels of ryegrass infestation which would have invalidated the treatment effects. From each plot, samples were taken from within two 10 m lengths of plot, one in the sandy loam and the other in the clay loam.

In each experimental unit, 20 random samples were taken with a trowel, removing about 50 to 100 cm^3 of soil at a time from the top 5 cm of soil. The soil samples were bulked in a plastic bag, mixed thoroughly by shaking, and kept at 5° until processing, three weeks later.

Soil inoculum densities (propagules per gram of soil [ppg]) of *B. sorokiniana* were estimated by soil plating on DR70 medium (Chapter 3). In the first year, a subsample of 20g was taken from each treatment and dried for 24 hours at 32°. Five dilution plates were made for each treatment, and counts of *B. sorokiniana* colonies were pooled for a treatment. In the second year, two 20 g subsamples were taken from

each plot sample and diluted separately. Three plates were made for both subsamples, giving six per plot, and the mean of these was calculated.

7.2.3. Data analysis

No variance was available from the alternate phase samples, as they were composites of replicates, so statistical analysis was not possible. Treatment means for the two years were compared using linear regression analysis. The fit of the regression indicated the extent to which the alternate phase data corroborated the wheat phase data.

The wheat phase data were analysed by ANOVA. Since two rotations were missing from the clay loam, a second ANOVA was performed with these rotations excluded. Results presented are from the first analysis except where stated.

7.3. Results

Treatment means for the alternate crop phase ranged from 8 to 120 ppg with a grand mean of 55 ± 28.4 ppg (Table 7.1). The fairly low standard deviation and the fact that some trends were apparent in the data (see below) indicated that, providing treatments were replicated to allow analysis, the sampling and plating methods were suitable for the second year.

Table 7.1. Effects of year, rotation, tillage practice and soil type on inoculum density of *B. sorokiniana* (propagules per gram of soil). Measurements were made using soil plating (Dodman and Reinke, 1982).

		Sandy loam			Clay loam			Both soils		
Rotation	Year	CC	DD	mean	CC	DD	mean	CC	DD	mean
Wheat	'87	120	36	78	56	32	44	88	34	61
/wheat	'88	225	156	191	179	111	145	202	133	168
Peas	'87	48	60	54	72	84	78	60	78	66
/wheat	'88	175	146	160	139*	122*	131†	157*	134*	146†
Medic	'87	76	32	54	32	20	26	54	26	40
/wheat	'88	160	90	125	101	64	83	131	77	104
Oats	'87	68	-	68	56	-	56	62	-	62
/wheat	'88	169	131§	150†	280¥	109§	121*	151	120	136†
Grass past.	'87	100	44	72	8	44	26	54	44	49
/wheat	'88	101	120	111	98	114	106	100	117	108
YEAR	'87	82	43	65	45	45	45	64	44	55
MEANS	'88	166	129†	147†	130†	104†	117†	148†	116†	132†

* plots not assessed (estimated value)
§ treatments not included in experiment (estimated value)
† means from estimated values
¥ outlier, estimated value = 133 (see text)

7.3.1. Years

Two points (grass/wheat*sandy loam*CC and oats/wheat*clay loam*CC) had high leverage on the regression of the second year's data on the first (Figure 7.1), but there were no obvious explanations for their inconsistencies. Because of the magnitude of their divergence from the trend, it is likely that they were the result of errors in sampling, storage or processing, so to describe the general case, they were removed from the analysis. Inoculum densities of treatments over the two years were highly correlated ($r^2 = 0.73$), showing that the alternate phase data strongly supported the wheat phase data (Table 7.1). Inoculum densities after the alternate phase were much lower than after the wheat phase (41% on grand means).

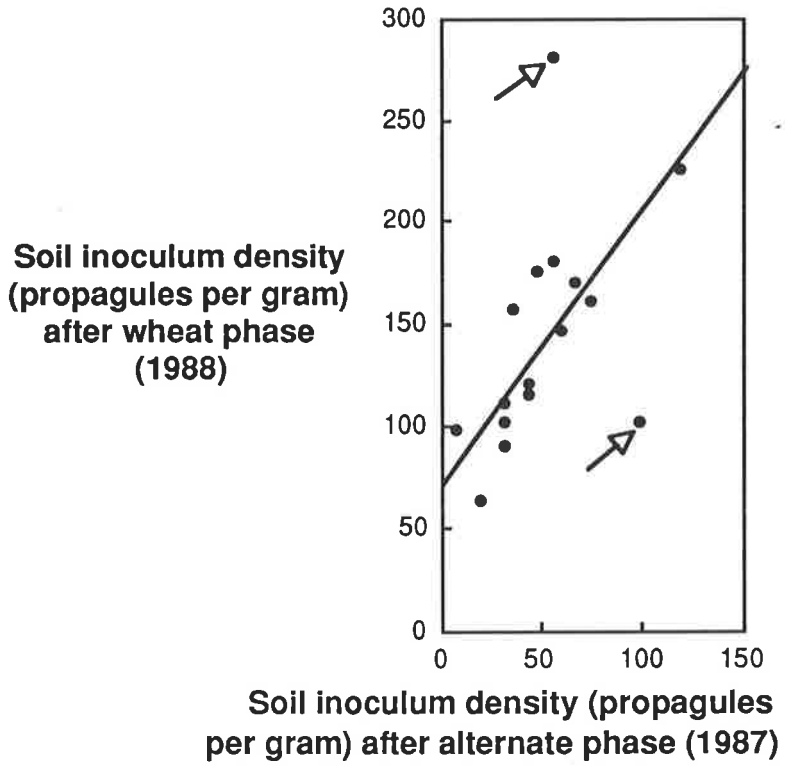


Figure 7.1. Comparison of soil inoculum densities (propagules per gram of soil) of *B. sorokiniana* in two years in a field trial with factorial treatments of rotations, tillage practices and soil types. Measurements were made using soil plating (Dodman and Reinke, 1982). The regression was calculated without the two outliers arrowed (see text).

$$y = 1.35x + 71.2,\ r^2 = 0.73\ (p < 0.001)$$

7.3.2. Rotations

Following the alternate phase in 1987 (Table 7.1), inoculum densities seemed to differ among rotations, although possible trends were obscured by apparent interactions between rotations, soil type and tillage practice. For example, peas had higher values than wheat in DD but not in CC. Grassy pasture values were similar to wheat in three samples, but much lower from clay loam*CC, than from the other treatments.

Following the wheat phase in 1988, inoculum density was significantly higher after continuous wheat than after medic/wheat and grassy pasture/wheat. The other

rotations were between these extremes and did not differ significantly from them ($p < 0.001$) (Table 7.2).

Table 7.2. Effects of rotations, after the wheat phase in 1988, on soil inoculum densities of *B. sorokiniana* (propagules per gram of soil). Measurements were made using soil plating (Dodman and Reinke, 1982). Means followed by the same letter are not significantly different by Tukey's HSD at $p < 0.05$.

Rotation	Inoculum density (ppg)
Wheat/wheat	168 a
Peas/wheat	146 ab
Oats/wheat	136 ab
Grass/wheat	108 b
Medic/wheat	104 b

7.3.3. Tillage Practices

In general, inoculum density was lower with DD than with CC (Table 7.1). In the first year, this was the case in the sandy loam but not the clay loam, with a 31% difference in the former. In the second year, inoculum density was significantly lower with DD than with CC on both soil types, by 34% ($p < 0.01$).

Inoculum density in the grassy pasture/wheat rotation in the first year was lower in DD than CC in the clay loam, but not in the sandy loam. In the second year, in the first analysis (peas and oats included), the interaction term of rotation*tillage practice was marginally significant ($p < 0.10$) and became significant when these rotations were removed from the analysis ($p < 0.05$). The grassy pasture/wheat rotation was unlike the others in having higher inoculum density with DD than with CC, although the difference between tillage practices was non-significant. There was no interaction with soil type to support the first year's observation. The grass/wheat*sandy loam*CC treatment was one of the two which was not consistent between years (Figure 7.1) but there was no clear reason for this.

7.3.4. Soil Type

Inoculum density was higher in the sandy loam by 31% after the alternate phase and 26% after the wheat phase ($p < 0.01$) (Table 7.1). Non-significant interactions of soil type with rotation and tillage practice have been described above.

7.4. Discussion

7.4.1. Suitability of methods used

The objective of the study was to determine whether the treatments representative of South Australian farming systems are or could be important in the control of common root rot. The trial was initiated in 1978, so it is likely that long term trends in soil inoculum density were established, and one sampling time should be sufficient to assess these trends.

Clearly, the time of sampling is important if the level of inoculum is to be related to disease levels in the crop, because inoculum density can change as a result of sporulation or germination, decline in conidial viability, or antagonism and parasitism (see Section 2.10.1). The optimum time for sampling which indicates the inoculum potential for crop infection. This may be shortly before or at sowing time as in the second year, when samples were taken about 4 to 6 weeks prior to the expected sowing date and prior to the opening rains. However, populations may be affected by tillage, herbicides and increased soil water potential in the period following sowing, so sampling in early spring, as done in the first year, may be more appropriate.

The population dynamics of *B. sorokiniana* have not been studied in an environment which resembles that of the South Australian cereal belt, but extrapolation from Canadian studies may be possible. Chinn (1965) observed a decline in soil inoculum density in wheat fields in the first few weeks of the growing season (spring), then a sharp increase in summer. Duczek (1990) found that this corresponded with the pattern of sporulation on plants. Tinline *et al.* (1988) also found soil inoculum density increased through the growing season at one site in Queensland, but not at two others.

There was a decline in inoculum density through summer in fallowed fields in Canada (Chinn, 1965). The sporulation pattern observed by Duczek (1990) was positively related to growth stage, cumulative soil temperature, cumulative degree days, time and cumulative precipitation. The importance of temperature in determining the rate of sporulation confirms the findings of Bailey *et al.* (1989) with disease progression studies, as discussed in Section 4.4.1. If so, soil inoculum density in South Australia could be expected to decline from harvest, in early summer, until the opening rains in late autumn, and possibly after sowing in early winter until spring, then infection and sporulation would increase with the onset of warmer weather (McKinney, 1923; Duczek, 1990).

7.4.2. Treatment effects

7.4.2.1. Years

There are two likely explanations for the differences in inoculum densities measured on the continuous wheat rotation between years. Firstly, the different sampling times could have had a substantial effect, reflecting population changes through the year as discussed above. Secondly, the population density changed between years, due to variation in temperature and precipitation. Both can directly affect parasitism by *B. sorokiniana* (McKinney, 1923; Sallans, 1948) and sporulation can vary by an order of magnitude (Duczek, 1990). Environmental fluctuations would also affect the competitive and antagonistic activity of other micro-organisms.

7.4.2.2. Rotations

The results support Butler's (1961) contention that alternating rotations will not remove common root rot, but can reduce its severity. As previously explained, common root rot levels were not measured in this study. However, the fact that different inoculum levels were maintained through the wheat phase in different rotations (Figure 7.1) constitutes evidence that rotations affected disease levels, since sporulation is probably proportional to the amount of tissue diseased.

The finding that continuous wheat led to the highest inoculum densities of all rotations is consistent with other reports (Ledingham, 1961; Chinn, 1976b; Reis and Wünsche, 1984; Wildermuth and MacNamara, 1991). Chinn (1976b) showed that inoculum density after wheat depended on the variety. Spear is less susceptible to common root rot than some other varieties (Chapter 6), and hence considerably higher inoculum densities could occur after continuous wheat of other varieties such as Machete.

It was also reported by Ledingham (1961) and Chinn (1976b) that oats led to lower inoculum density than wheat, although Ledingham (1961) showed a greater difference than that observed in the Avon soils, and in the studies of Wildermuth and MacNamara (1991), inoculum density after oats was not significantly different from that after wheat. These different results may have arisen through differences in the relative resistance of oat and wheat varieties used in the different studies.

There are no other reports on the effect of peas on soil inoculum density of *B. sorokiniana*, although Malikova (1968, cited in Piening and Orr, 1988) found less root rot in wheat after peas than after wheat or fallow. Wildermuth and McNamara (1987) found peas to be moderately susceptible to infection, although they did not report data on sporulation. The findings of Reis and Wünsche (1984) with other leguminous crops suggest that infection on peas may lead to a sporulation rate similar to that on wheat, although among the legumes tested in rotation with wheat by Wildermuth and MacNamara (1991), all led to significantly lower inoculum densities than in continuous wheat.

The medic species in the Avon medic pasture (*M. scutellata*) apparently has not been tested for its effect on inoculum density, although Wildermuth and McNamara (1987) showed it could become heavily infected. The relatively low inoculum density following medic at Avon indicated that little sporulation had occurred on this species so measurements of infection alone did not indicate its value as a break crop for common root rot.

7.4.2.3. Tillage Practices

The general finding of lower inoculum with DD than CC supports that of Reis and Abrão (1983), who found 35% less inoculum in DD than in CC in the top 5 cm of soil. They also found greater vertical dispersal of inoculum after CC than after DD.

In the Avon trial, both CC and DD treatments were sown shallowly, but in practice in South Australia, CC crops are often sown deeper than DD. Broadfoot (1934) suggested that shallow sowing might reduce common root rot and this was supported subsequently by several reports (Greaney, 1946; Tinline, 1986; Broscious and Frank, 1986; Piening *et al.,* 1969; and Duczek and Piening, 1982).

Samples in the present study were taken irrespective of drill row position, so the relative contributions to inoculum density of samples from between and in drill rows were not measured. In farm crops, the differences in soil disturbance between CC and DD may lead to lower inoculum levels in the latter. Tillage in CC would distribute inoculum between rows, whereas in DD, inoculum may remain associated with plant crowns. Generally, rows would be randomly placed (although parallel) between years, so that in DD, plants may escape areas of significant inoculum density. This would not have been the case in the Avon trial, since rows are coincident between years. Deliberately sowing crops in the inter-row spaces of preceding crops might help to control this disease. Accurate placement of rows might be easier with patterns such as two rows close together with a wide gap before the next pair, than with evenly spaced rows. Such patterns are being tested for improved placement of herbicides and fertilisers.

These results only apply to DD with minimal soil disturbance and not to "minimum tillage", since one cultivation might disperse inoculum as much as several. Conner *et al.* (1987) compared common root rot ratings in minimum tillage plots and CC plots. Less disease occurred in the former in two of 11 years, but ratings were higher in one year. Inoculum levels were not reported by these workers.

A difference in inoculum density between DD and CC might not occur where infection and extensive sporulation occurred above the crown, resulting in wider passive dispersal than with soilborne inoculum alone. However, while there are anecdotes of sporulation of *B. sorokiniana* on stubble in South Australia (A.J. Rathjen, personal communication), there are no formal records of leaf infection in this state, where spring humidity (after crop tillering) is usually low.

A possible explanation for the significant interaction between tillage practice and rotation (higher inoculum density in DD than in CC in the grass/wheat rotation) is pasture composition. Ryegrass (*L. rigidum*) dominates the pasture in CC at the Avon trial site, whereas barleygrass (*H. glaucum)* dominates in DD (D.K. Roget, personal communication). The result indicates that *H. glaucum* may be more susceptible to *B. sorokiniana* than *L. rigidum*. Christensen (1922) tested neither species, but showed differences in susceptibility among species in both genera. Reis and Wünsche (1984) found that soil inoculum density was higher after pure *L. multiflorum* than after wheat, although the reverse was true with a mixed medic/*L. multiflorum* pasture.

7.4.2.4. Soil type

This is the first report from an experiment, rather than a survey, of soil type apparently affecting inoculum potential of *B. sorokiniana* under identical climatic and cropping regimes. That inoculum density tended to be higher in the sandy loam than the clay loam corroborates the report of Samuel (1924) that infections by *B. sorokiniana*, while common in wheat on sandhills, were not found on the heavier soils of the adjacent flats.

One hypothesis for sandy loam having higher inoculum density than clay loam concerns dispersal. Tillage may move the spores around much more freely in a loose, unstructured soil. If this was the case, an interaction with tillage practice would be expected, *viz* the DD value would be the same on both soils. There was no significant interaction between these factors, although inoculum density in the second year in DD*sandy loam was 19% higher than in DD*clay loam.

The difference between soil types in inoculum density may be related to water availability. The soils differ in their moisture characteristics, and may also have differed in water availability because of their locations (the clay loam being in the swale where water would collect, the sandy loam being on the slope, where water would run off). Water potential was not monitored through the experiment, and there is insufficient knowledge of the effects of water potential on infection and sporulation of *B. sorokiniana* to support speculation. Other factors which vary between soil types and which may have contributed to the differences include nutritional status, organic matter level, weed, microflora and microfauna spectra and soil bulk density. Given the importance of these two soil types in South Australia, further studies may be warranted on their effects on inoculum density of *B. sorokiniana*.

7.4.3. General

Reducing inoculum density of *B. sorokiniana* might not affect disease parameters if the density remains above that which causes maximum disease levels (Section 2.10). However, since the different rotations with wheat led to a range of inoculum densities, it can be inferred that disease levels varied between rotations. Therefore, in this experiment, the three factors (rotation, tillage and soil type) seemed to have agricultural importance in their effects on both *B. sorokiniana* and common root rot.

Elsewhere in this thesis (Chapter 4), it was shown that common root rot varied in prevalence between regions and it was hypothesised that the disease was affected by soil types, by different rotations prevalent in the regions, and by wheat varieties varying in resistance to common root rot. The data here provide evidence supporting those hypotheses.

That inoculum density being lower on clay loam than sandy loam has practical significance. On clay loams, there may be less risk in sowing crops such as barley and wheat varieties susceptible to common root rot. On the other hand, farmers on sandy loams may need to exercise greater care and use alternative crops, common root rot-resistant wheat varieties and a systemic fungicidal seed-dressing which reduces

common root rot. The lower inoculum potential under DD than CC may be another reason (in addition to soil conservation) for farmers on light soil types to adopt direct drilling.

8. EFFECTS OF FUNGICIDES ON COMMON ROOT ROT

8.1. Introduction

The use of fungicidal seed-treatments is recommended for all seed for South Australian wheat and barley crops (Wallwork, 1991), to prevent contamination of grain with smut fungi and to control some foliar diseases. The recommendation is adopted for almost all crops, due to strict standards for receival/rejection of grain and discounts for smut contamination.

At the time of this study, no fungicides were registered in the state for control of common root rot. It was considered important for two reasons to determine the efficacy against common root rot of the major fungicides used in South Australia. Firstly, a fungicide controlling common root rot as well as its current target diseases may be preferable to other fungicides. Secondly, a fungicide which controlled common root rot might be useful in quantifying the effects of this disease on crop yields.

In a preliminary experiment with fungicidal seed-dressings (A.J. Rathjen, A.J. Dubé and J. Lewis, unpublished data), flutriafol (Armour® and Vincit®, ICI Australia Ltd.) applied by liquid seed dressing substantially reduced symptoms of common root rot, although it was applied at a high rate which was phytotoxic and reduced yield. Triadimenol (Baytan®, Bayer (Aust.) Ltd.) applied as a dry powder dressing did not reduce common root rot. Scheinpflug and Duben (1988), in reviewing studies on the modes of action of fungicides, stated triadimenol to be active against soil-borne *B. sorokiniana* and other authors have found similarly (See Section 2.11), but the activity of flutriafol was not reported. Consequently, further experiments were conducted with flutriafol and triadimenol, both as seed-dressings.

Experiments were also conducted with fertilisers amended with flutriafol and triadimefon (Bayleton®, Bayer (Aust.) Ltd.). Because these fungicides, like other triazoles, are translocated acropetally only (Scheinpflug and Duben, 1988), application by this method may give better control of this disease than seed-dressings

through allowing uptake of fungicide by roots. Furthermore, less phytotoxicity occurs with this method than with seed-dressing, and the fungicides are detectable in the plant for much more of the growing season (D. Ballinger, personal communication).

8.2. General Methods

Seedling emergence, or plant stand density, was determined usually at about 6 to 8 weeks after sowing, by counting seedlings within two randomly placed 50 cm x 4 row quadrats in each plot.

Effects of treatments were analysed with ANOVA. In experiments involving several rates of a fungicide, the shape of the dose response was investigated by fitting orthogonal polynomials to the data using the POL function in GENSTAT 5©. The regression equation of the highest order polynomial, up to cubic, which was found to be significant by F-test was then calculated from the means for each fungicide rate.

8.3. Experiments

8.3.1. Synopsis of experiments

Experiments with Fungicidal Seed-dressings

Experiment 1.

Flutriafol was applied as a seed-dressing at five rates to two wheat varieties in a field experiment in 1987. Effects on common root rot, plant growth and yield were determined.

Experiment 2.

Treated seed from Experiment 1 was germinated and grown in moist paper rolls to test effects of flutriafol on early growth of two wheat varieties.

Experiment 3.

Five fungicides which were commercially available or pending registration were applied to wheat and barley, in a field experiment in 1989. Their effects on common root rot, and infection by *B. sorokiniana* were examined.

Experiments with Fungicide-amended Fertilisers

Experiment 4.

Flutriafol and triadimefon were applied as fertiliser-amendments to two varieties of wheat in the field in 1985 and their effects on common root rot and yield were assessed.

Experiment 5.

Flutriafol was applied as fertiliser-amendment at five rates to three varieties each of wheat and barley, in the field in 1987. Effects on common root rot, plant growth and inoculum density of *B. sorokiniana* were tested.

8.3.2. Experiment 1.

Effects of flutriafol applied as seed-dressing for control of common root rot of wheat in the field in 1987.

8.3.2.1. Materials and Methods

The effects of flutriafol as a seed-dressing at different rates were examined in a field experiment in 1987. Two varieties of wheat, Kite and Machete, respectively moderately resistant and susceptible to common root rot (Table 6.3), were treated with five rates of flutriafol. Treatments were arranged in a split plot design with variety as wholeplots and fungicide rate as subplots, with 20 replicates, in order to be able to detect small yield responses.

Seed was treated with four rates of Armour (dry powder, 10% flutriafol, 0.4% cypermethrin insecticide) in an ICI/Link motorised spinning drum seed-treater. Seed in 250 g lots was placed in the drum and the required quantity of fungicide was dusted on top. The seed was mixed for two minutes, during which the drum was inverted several times. The control seed was mixed without fungicide, then four batches were mixed successively to give nominal rates of 0, 50, 100, 150 and 200 ppm of flutriafol. Residues remaining in the drum after treatments were wiped out with ethanol between each batch. As not all fungicide became attached to the seed, actual rates were below nominal rates, but the discrepancies were not quantified.

The experiment was sown at Mannum, on the property of Mr. B. Ramm (site details as for Experiment W781/1987, Table 6.1). The soil type was red sand at the surface, pH 8, grading to sandy clay, pH 10, at 50 cm depth. The rotation was grassy pasture in 1985 and wheat in 1986. The trial was sown on 30 May. Seed was placed deep (6 to 8 cm) to induce long subcrown internodes to form, to enable scoring of common root rot. To control cereal cyst nematode (*H. avenae*) which was present on the 1986 wheat crop, Temik 15G (15 g/kg aldicarb) was applied through the cones into the furrow with the seed at 8 kg/ha (120 g a.i./ha).

Emergence was assessed on 17 June (18 days after sowing) before all plants had emerged so the count was of rate of emergence, rather than final stand density.

Common root rot on subcrown internodes was assessed twice, first at tillering (Zadoks stage 20 to 25) for incidence on 4 August (10 weeks after sowing) and then at maturity (Zadoks stage 91) for incidence and severity.

On 26 October (21 weeks after sowing, at milk, Zadoks stage 70) it was observed that leaf tips were bronzed and flag leaves were becoming necrotic, and that this varied between plots. All plots were scored for flag leaf necrosis, on a 0 to 5 scale, with intervals corresponding to 20% of the leaf area being necrotic. Deadheads (prematurely ripened heads with no grain) were counted at this time (full plot counts) and tiller density was counted over two 50 cm lengths of row in each plot (tillers per m).

The trial was harvested when mature and plot grain yields were measured.

Effects of the factors Variety and Fungicide on the variates were analysed.

8.3.2.2. Results and Discussion

1. Effects of flutriafol on common root rot

Common root rot symptoms were found on 64.1% of untreated plants at tillering. Disease incidence was reduced by fungicide application ($p < 0.001$), by 65 % with 50 ppm of flutriafol, but no further with greater rates (Figure 8.1). The cubic regression was significant ($p < 0.01$, $r^2 = 0.96$).

The two varieties, which differ in resistance to common root rot, were not shown to be different at this sampling time.

The maturity disease scores reflected the tillering scores, with disease incidence being reduced significantly by fungicide ($p < 0.001$, Figure 8.2). The quadratic regression was highly significant ($p < 0.001$, $r^2 = 0.99$). Efficacy of flutriafol improved only

slightly at rates greater than 50 ppm. Incidence in the control (79.6%) had increased from the earlier sampling, but the difference between the control and the 50 ppm treatment had narrowed to 25.4%, as a result of further disease development in the treated plots.

There was 22.6% higher incidence on Machete (64.9%) than on Kite (52.9%) ($p < 0.001$) at maturity, whereas the varieties did not differ in the first sampling. This phenomenon was described by Stack (1981) and Bailey *et al.* (1989); differences in resistance only became evident after the log phase of increase in the frequency of disease symptoms.

Dose response at maturity of severity of common root rot was similar to that of disease incidence (Figure 8.3), with a 39% reduction at 50 ppm ($p < 0.001$). This was described by a quadratic relationship ($p < 0.001$, $r^2 = 0.97$).

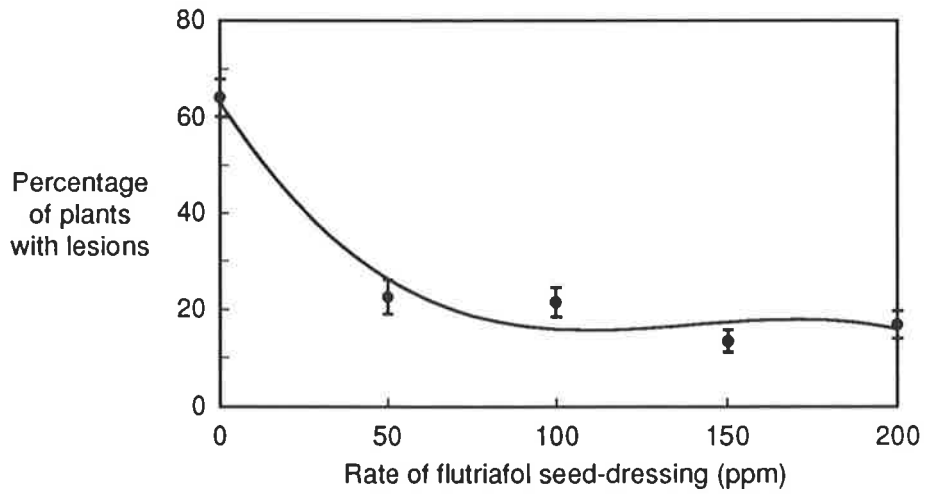


Figure 8.1. Effect of five rates of flutriafol seed-dressing on incidence of common root rot on wheat 10 weeks after sowing at Mannum in 1987. Error bars are standard errors, n = 40.
$y = 63.15 - 1.10x + 0.008\ x^2 - 0.0002x^3$, $r^2 = 0.96$.

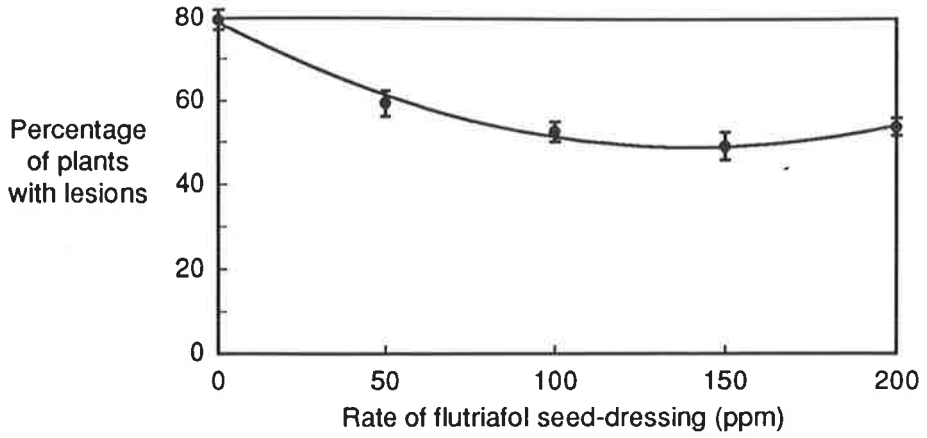


Figure 8.2. Effect of five rates of flutriafol seed-dressing on incidence of common root rot on wheat at maturity at Mannum in 1987. Error bars are standard errors, n = 40.

$y = 78.9 - 0.43x + 0.002x^2$, $r^2 = 0.99$.

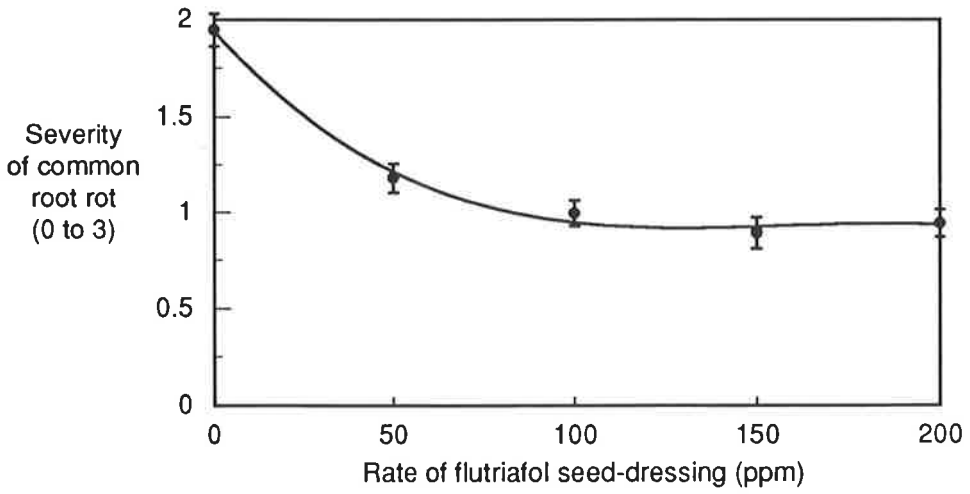


Figure 8.3. Effect of five rates of flutriafol seed-dressing on severity of common root rot on wheat at maturity at Mannum, 1987. Error bars are standard errors, n = 40.

$y = 1.90 - 0.91x + 0.0001x^2$, $r^2 = 0.97$.

2. Effects of flutriafol on plants

For both varieties of wheat, emergence was negatively associated with flutriafol rate ($p < 0.05$), but the sensitivity of Machete was slightly greater (e.g. 34% reduction from 100 ppm from the regression, c.f. 26% for Kite) ($r^2 = 0.97$ and 0.99 respectively) (Figure 8.4), possibly due to the shorter coleoptile of Machete (Experiment 2).

Flutriafol significantly reduced the fresh weight of plants sampled at tillering (Figure 8.5), with a significant linear regression of plant weight on fungicide rate ($p < 0.001$, $r^2 = 0.82$), the reduction being 20% at 200 ppm, calculated from the regression.

The phytotoxic effects of flutriafol evident in emergence and in plant fresh weight at tillering were also evident at maturity, as a reduction in tiller density ($p < 0.001$, Figure 8.6) with a linear trend ($p < 0.001$, $r^2 = 0.99$). From the regression, 100 ppm of flutriafol reduced tiller density by 11%. The two varieties differed significantly ($p < 0.05$) but the difference was small (5.7%).

Increasing rates of fungicide application decreased flag leaf necrosis in Machete, and high rates had the same effect on Kite ($p < 0.001$, Figure 8.7). Kite had less necrosis at all rates than Machete. The quadratic response of the interaction was significant ($p < 0.05$), Machete being better represented by the linear model ($r^2 = 0.95$) and Kite by the quadratic ($r^2 = 0.97$). Variation in necrosis could be due to effects of the fungicide on disease, and/or to plant maturity being delayed by the fungicide. The curves (Figure 8.7) are similar to the curves for emergence (Figure 8.4), plant fresh weight at tillering (Figure 8.5) and tiller density (Figure 8.6) but dissimilar to those for disease (Figures 8.1, 8.2 and 8.3), so the latter explanation is more probably correct. The polynomial response observed in Kite could have arisen from an interaction of plant growth stage and environment.

Deadhead density differed significantly between varieties (Kite 7.00, Machete 11.32 deadheads per plot) ($p < 0.001$), and fungicide rate ($p < 0.01$) with the quadratic model providing the best description ($p < 0.001$, $r^2 = 0.91$) (Figure 8.8). The increase

and then decline with increasing fungicide rate could be due to combined effects of disease control and delayed maturity and, like flag leaf necrosis, deadhead density could be an indirect outcome of both effects.

Grain yield was significantly reduced by flutriafol application ($p < 0.001$), being reduced linearly with increasing rate ($p < 0.001$, $r^2 = 0.90$) (Figure 8.9). The loss was 27% at 200 ppm, calculated from the regression.

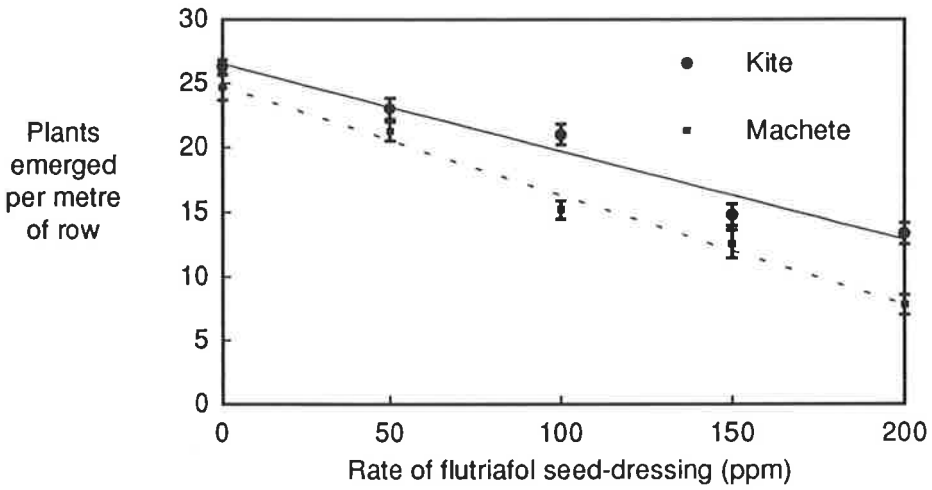


Figure 8.4. Effect of five rates of flutriafol seed-dressing on emergence of two varieties of wheat at Mannum, 1987. Error bars are standard errors, n = 40.
y (Kite) = 26.46 - 0.068x, $r^2 = 0.97$.
y (Machete) = 24.75 - 0.085x, $r^2 = 0.99$.

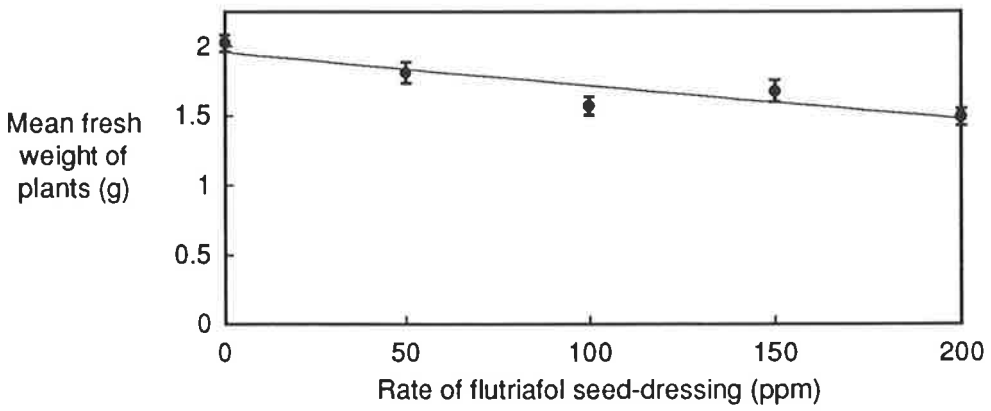


Figure 8.5. Effect of five rates of flutriafol seed-dressing on fresh weight of wheat plants at tillering, at Mannum, 1987. Error bars are standard errors for means over both varieties, n = 40.
y = 1.957 - 0.002x, $r^2 = 0.82$.

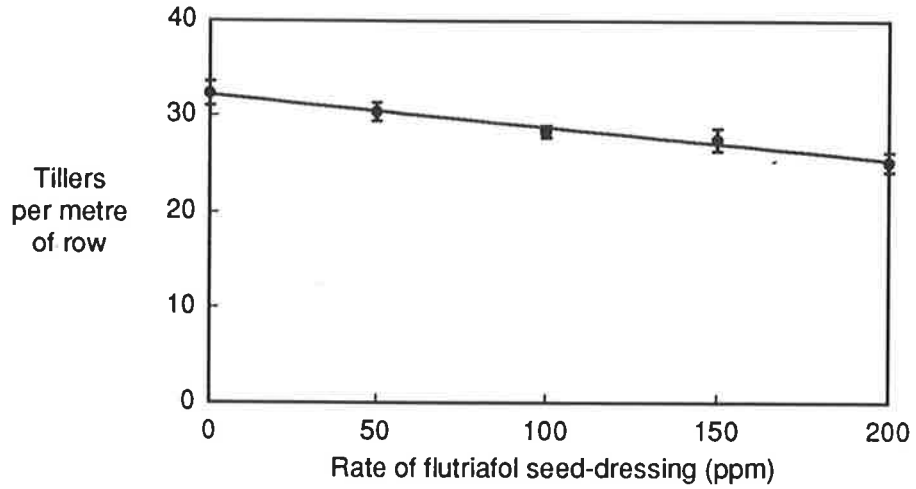


Figure 8.6. Effect of five rates of flutriafol applied as seed-dressing on number of tillers of wheat per metre of row, at Mannum, 1987. Error bars are standard errors.

$y = 32.13 - 0.034x$, $r^2 = 0.99$.

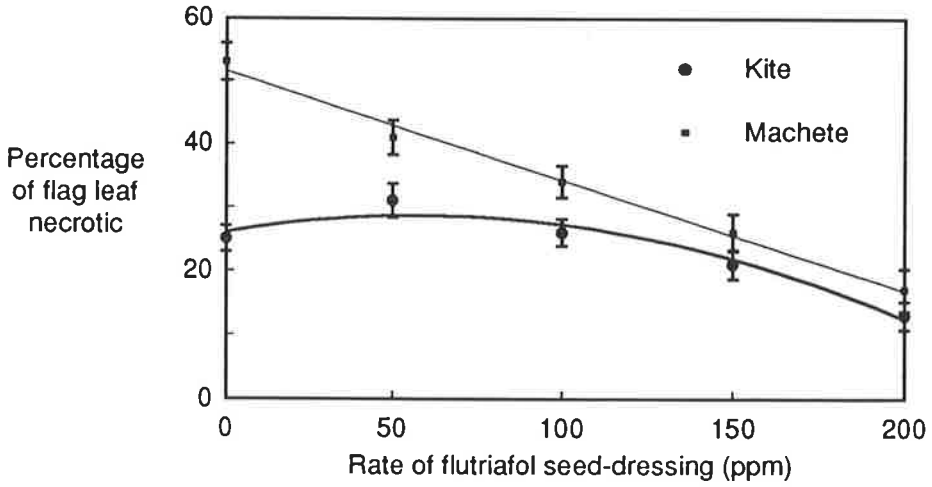


Figure 8.7. Effect of five rates of flutriafol seed-dressing on necrosis of flag leaves of two wheat varieties, Mannum, 1987. Error bars are standard errors, n = 40.

y (Kite) = $26 + 0.092x - 0.001x^2$, $r^2 = 0.95$.

y(Machete) = $51.6 - 0.174x$, $r^2 = 0.99$.

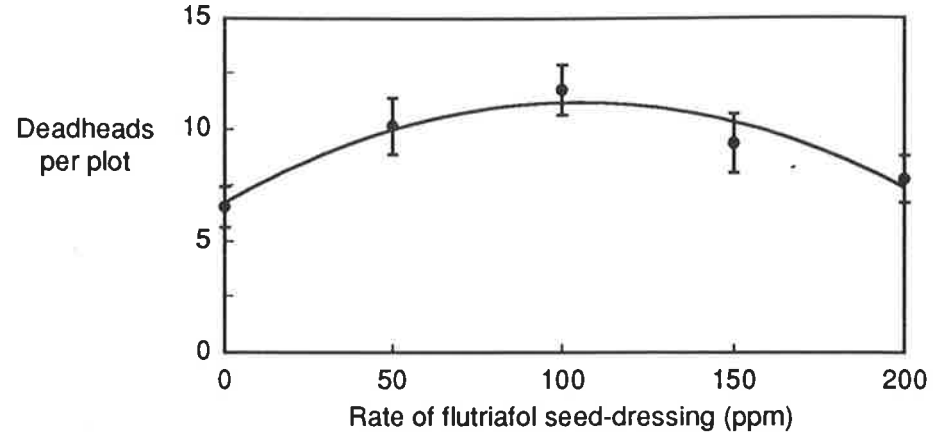


Figure 8.8. Effect of five rates of flutriafol seed-dressing on density of deadheads in wheat at Mannum, 1987. Error bars are standard errors, n = 40.
$y = 6.73 + 0.087x - 0.0004x^2$, $r^2 = 0.91$.

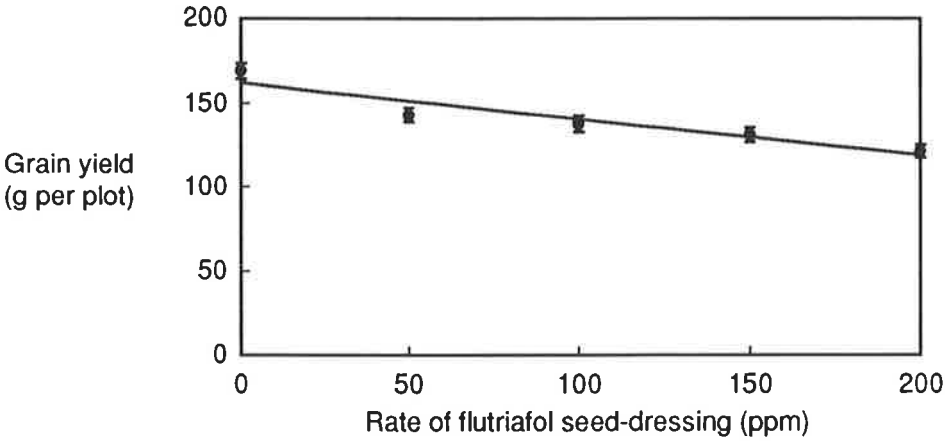


Figure 8.9. Effect of five rates of flutriafol seed-dressing on grain yield of wheat at Mannum, 1987. Error bars are standard errors, n = 40.
$y = 162.0 - 0.22x$, $r^2 = 0.90$.

8.3.3. Experiment 2. Effects of flutriafol, applied as seed-dressing, on coleoptile lengths of two varieties of wheat grown in moist paper rolls.

8.3.3.1. Materials and Methods

The preliminary experiment in 1984 had indicated flutriafol to be potentially phytotoxic and further evidence was found in Experiment 1. This was investigated using samples of the fungicide-treated seed from all treatments of Experiment 1, five months after the seed was treated. Sheets of filter paper (Ekwip Grade R6) were moistened with reverse osmosis water and drained. For each treatment, 25 seeds were placed in a line across the paper, 5 cm from the base edge, and the paper was rolled up. Four replicates were made. Rolls were wrapped individually in aluminium foil, leaving the ends open, stood on their bases in a wire rack and incubated at 15° for 13 days. The rolls were opened, coleoptile lengths were measured and mean lengths were calculated for each roll.

8.3.3.2. Results and Discussion

Coleoptiles were significantly reduced in length by flutriafol application. There was a significant interaction of Variety*Fungicide ($p < 0.001$) from the cubic model ($p < 0.001$) (Figure 8.10). The inherent coleoptile length (with no fungicide) of Kite was greater than that of Machete. At 50 ppm of flutriafol, more reduction occurred in Kite than in Machete, but even at 100 ppm, Kite formed a coleoptile only slightly shorter than Machete in the control treatment. The magnitude of reduction at 50 and 100 ppm in both varieties corroborates the findings in Experiment 1, and indicates that substantial care is needed in using this fungicide commercially, to ensure control of sowing depth.

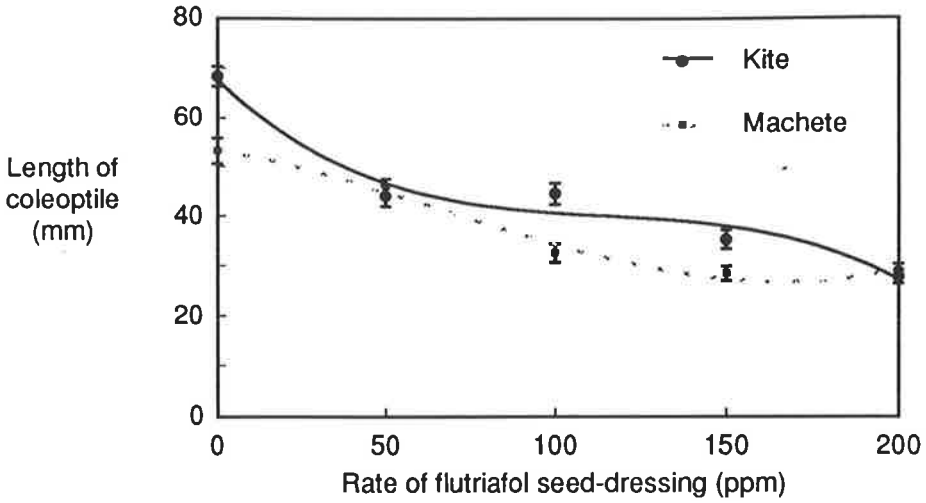


Figure 8.10. Effect of five rates of flutriafol seed-dressing on coleoptile length of two wheat varieties grown in paper rolls. Error bars are standard errors, n = 40.
y (Kite) = 67.9 - 0.65x + 0.005x^2 - 0.00001x^3, r^2 = 0.96.
y (Machete) = 53.6 - 0.14x - 0.001x^2 + 0.0.00006x^3, r2 = 0.99.

8.3.4. Experiment 3.

Comparison of flutriafol, triadimenol and three other fungicides applied as seed-dressings to barley and wheat for control of common root rot.

8.3.4.1. Materials and Methods

Five seed-dressing fungicides were tested for efficacy against common root rot, in a field experiment on wheat and barley in 1989 (Table 8.1). Only fungicides which were commercially available or pending registration were included, and all were systemic, dry powder formulations. Seed for each treatment was treated in several batches and then bulked. Seed was treated in an ICI/Link motorised spinning drum seed-treater (see Section 8.3.2.1). The control seed was treated in this way, then the fungicide treatments were done. Residues remained in the drum after treatments, and these were wiped out with ethanol between each batch. Thus, actual rates were below nominal rates, although the discrepancies were not quantified.

Table 8.1. Seed-dressing fungicides tested for efficacy against common root rot on wheat and barley at Windsor, Geranium and Mudamuckla in 1989.

Product	Formulation	Nominal rate (g a.i./ 100 kg seed)
Armour C	flutriafol 100 g/kg, cypermethrin 4 g/kg	100
Vincit C	flutriafol 50 g/kg, cypermethrin 4 g/kg	50
Vitavax 750	carboxin 750 g/kg	937.5
Baytan 100	triadimenol 100 g/kg	100
Raxil	tebuconazole 25 g/kg	25

One variety each of wheat and barley were treated, Machete and Schooner respectively, both susceptible to common root rot (Chapter 6). Wheat variety Schomburgk (moderately susceptible) was included as an untreated check. The experimental design was a replicated (x7) split plot with variety as main plots. Thus, each wholeplot had 5 fungicide-treated plots, a control and the check variety. Trials were sown at three sites, near the towns of Windsor, Geranium and Mudamuckla, at

which high levels of common root rot had been observed in previous years. The soil at Windsor was a brown calcareous sandy loam, pH of 8.5 to 9.5. The field was sown to wheat in 1988. The soil at Geranium was a grey siliceous sandy soil, pH 8.5 to 9.5, and grew a grassy pasture in 1988. The soil at Mudamuckla was a brown calcareous sandy loam, pH of 8.5 to 9.5, and grew a grassy pasture in 1988. The trials were sown on 10 April, 26 May and 7 June for Mudamuckla, Windsor and Geranium respectively. The inoculum density of *B. sorokiniana,* measured by soil plating on DR70 medium at Mudamuckla on 10 April, prior to the opening rains, was 72 spores per gram of soil. Inoculum density at the other sites was tested after sowing, on 12 July, and was 55 and 25 spores per gram of soil at Windsor and Geranium respectively.

Emergence was scored at Windsor and Geranium after six weeks. The Mudamuckla trial was to be measured only for disease levels at maturity and grain yield, due to its remoteness from the laboratory in Adelaide.

The rates of progress of common root rot severity and rate of infection by *B. sorokiniana* were measured and are reported in Sections 4.2.3 and 5.2.2 respectively.

Common root rot scoring was commenced at early dough (Zadoks stage 83) at Windsor on 10 October, but only on Control, Armour C, Baytan 100 and Schomburgk plots, since during earlier site monitoring there had been no indication that Vitavax 750 and Vincit C had reduced common root rot. However, scoring was abandoned due to severe distortion of subcrown internodes treated with Armour C and Baytan 100. They tended to be short and thick, hard and woody, and bright and clean in appearance, with the crown formed unusually deeply relative to the soil surface. Disease was not scored at Geranium, since early monitoring indicated negligible disease levels.

Sampling was commenced at early dough (Zadoks stage 83) on wheat and barley at Mudamuckla, but was discontinued on wheat, due to distortion of the subcrown

internodes. However, distortion of barley was less severe, so scoring was continued on it, on 35 randomly chosen plants per plot.

The effect of Armour C and Baytan 100 on isolation frequency of *B. sorokiniana* was determined on samples of barley taken from Windsor and Geranium on 1 December. This was not done for wheat, due to distortion of subcrown internodes. About 50 plants were removed at random from the plots of the two treatments and the controls. Subcrown internodes were removed, surface sterilised (Chapter 3) and plated in water agar/DR70 sandwiches (Section 5.2.2). Isolation frequency data were analysed with by regression analysis, assuming a binomial data distribution. Replicate totals of subcrown internodes yielding colonies and subcrown internodes plated were used. Replicate, treatment and site were fitted to the model for comparison for isolation frequency.

8.3.4.2. Results and Discussion

Emergence was not significantly affected by any of the fungicides at Windsor and Geranium.

At Mudamuckla, consistent with the finding in the preliminary experiment in 1984, Armour C reduced common root rot severity and Baytan 100 did not, although this difference was not statistically significant (Figure 8.11). The more resistant wheat check, Schomburgk, had significantly less disease than barley treated with Baytan 100 and the barley control ($p < 0.05$). While severity was apparently reduced by Armour C, it was still higher than on Schomburgk.

The two sites, Windsor and Geranium, did not differ significantly for isolation frequency of *B. sorokiniana* from barley subcrown internodes, but there were significant differences between treatments ($p < 0.001$) (Figure 8.12), with infection in the Armour C treatment being much lower than in Baytan 100 and control, which did not significantly differ. This was consistent with severity levels in the treatments at Mudamuckla and also in Experiment 1 and the preliminary experiment with wheat. It

also supports the findings in Chapter 5 on causation of common root rot by *B. sorokiniana*.

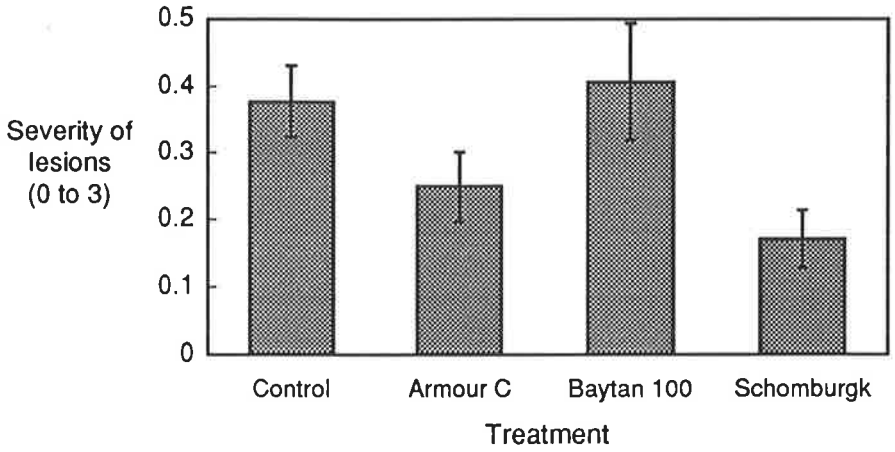


Figure 8.11. Severity of common root rot lesions on subcrown internodes of Schooner barley treated with the seed-dressing fungicides Armour C and Baytan 100, compared with a control and the more resistant wheat check variety Schomburgk, at Mudamuckla, 1989. Error bars are standard errors, n = 7.

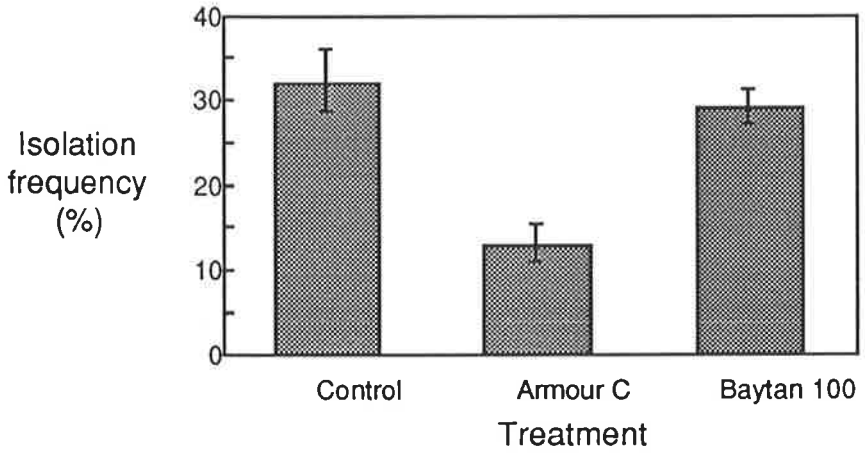


Figure 8.12. Isolation frequency of *B. sorokiniana* from subcrown internodes of barley treated with Armour C and Baytan 100 seed-dressing fungicides. Data are from two sites which were not significantly different. Plants were sampled at milky dough stage. Error bars are standard errors, n = 14.

8.3.5. Experiment 4.

Comparison of flutriafol and triadimefon applied as fertiliser-amendments to two varieties of wheat for control of common root rot in the field in 1985.

8.3.5.1. Materials and Methods

Flutriafol and triadimefon applied as fertiliser-amendments were tested in the field in 1985 for efficacy against common root rot on wheat. The experiment was conducted at Streaky Bay, on the property of Mr. B. Williams, on a highly alkaline grey calcareous sand. The rotation was grassy pasture in 1984 and wheat in 1983. The trial was sown on 18 June, into dry soil. Subsequent rainfall was inadequate (April to October rainfall was 110 mm at Streaky Bay) and resulted in moisture stress on the crop.

Two wheat varieties were used, Machete and Miling, both susceptible to common root rot (Table 6.3). Fungicide-amended fertiliser was supplied by ICI Australia Ltd. (flutriafol) and Bayer (Aust.) Ltd. (triadimefon). Pivot double superphosphate (16.2% phosphate), was treated with fungicide to contain triadimefon at 243 g a.i./ha and flutriafol at 250 g a.i./ha when applied at 50.7 kg/ha, and the unamended fertiliser was used as the control. A replicated (x8) split-plot design was used, with variety as wholeplots and fungicide treatment as subplots. Plots were sown by the SA Department of Agriculture regional agronomy group, using a Wintersteiger coneseeder, at 5 cm depth. Seed and treated or control fertiliser was sown through the cones together.

Common root rot severity was scored at anthesis (Zadoks growth stage 61 to 69). Plots were harvested for grain yield.

8.3.5.2. Results and Discussion

The two wheat varieties did not differ significantly in incidence or severity of common root rot. Incidence and severity from the pooled variety data were

significantly reduced by flutriafol, (eg 49% of the control value for incidence) ($p < 0.001$), but triadimefon had no effect (Figure 8.13), as observed with application by seed-dressing in the preliminary experiment in 1984 and in Experiment 3.

Yields were very low (control mean 280 kg/ha) due to drought. Yield was reduced by triadimefon by 16.8% ($p < 0.05$), and by flutriafol by 11.4% (not significant).

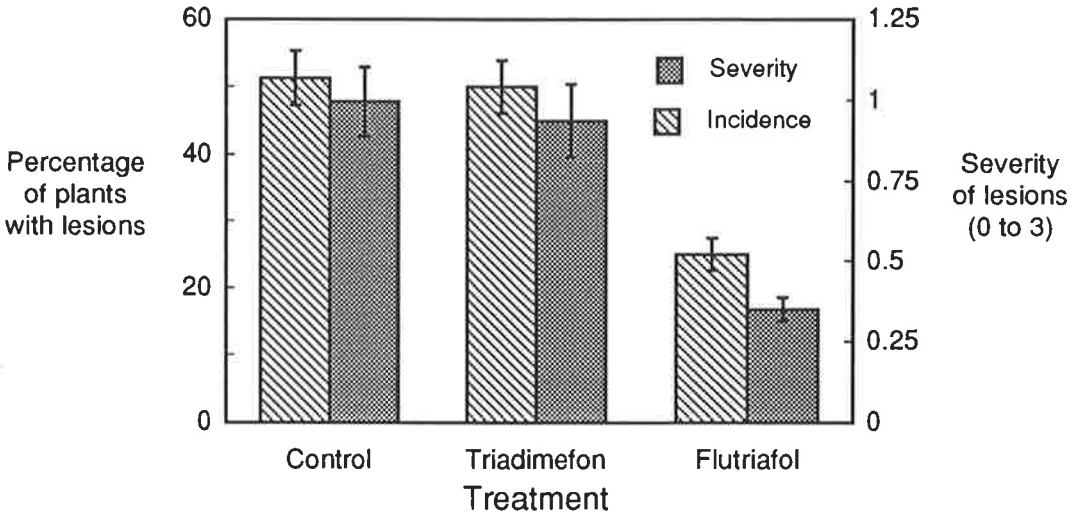


Figure 8.13. Effects on incidence of common root rot on wheat, of fungicides applied in the furrow as fertiliser-amendments, Streaky Bay, 1985. Incidence means are detransformed. Error bars are standard errors, n = 16.

8.3.6. Experiment 5.

Effects of flutriafol applied as fertiliser-amendment at five rates to three varieties each of wheat and barley for control of common root rot in 1987.

8.3.6.1. Materials and Methods

Because of the reduction of common root rot in wheat with flutriafol applied as a fertiliser-amendment in Experiment 4, a further experiment with this treatment was conducted in 1987, in which five rates of fungicide were applied to three varieties each of wheat and barley.

Flutriafol was applied in a field experiment at five rates, 0, 52.5, 85.5, 136.5 and 190.5 g a.i./ha. Topfos double superphosphate (16.2% phosphate) was mixed with Armour (25% flutriafol DS) powder in a tumbling mixer in 3 kg batches, for 5 minutes each. Batches were prepared to give nominal treatment rates of 0, 50, 100, 150 and 200 g a.i./ha. Treatments, including control, were mixed in order of increasing fungicide rate, and the drum was wiped out with ethanol to remove unattached fungicide after each treatment. Final flutriafol concentrations (above) were determined by testing 250 g subsamples of each treatment by gas chromatography (Appendix 2).

The wheat varieties were Machete, Warigal and Aroona, rated as susceptible, moderately susceptible and moderately resistant (Table 6.3). Barleys were Forrest, Galleon and Schooner, the former being among the most susceptible and the latter two among the least susceptible (Table 6.7).

Wheat and barley trials were sown end-to-end with each other, in randomised complete block designs with seven replicates. Different randomisations were used at three sites, Mannum, Sanderston Airstrip and Balaklava 110 (details as for Experiment W781/1987, Table 6.1). Soil population densities of *B. sorokiniana* in samples taken on 1 May were 40, 83 and 85 propagules per gram respectively. The

sites were sown on 25 May, 25 May and 2 June respectively. Treated fertiliser and seed were sown through the cones into the furrow together.

Samples were taken for common root rot scoring near maturity on 9 November at Mannum, on 13 November at Balaklava and on 12 November at Sanderston. About 15 plants were uprooted at random from each plot and later scored for severity of common root rot lesions on the subcrown internode. At Mannum, deadheads (prematurely ripened heads with no grain) were common and so were counted in each plot on 26 October (milk, Zadoks stage 70).

In the following autumn on 14 March, 1988, soil was sampled from each plot of the Mannum trial to determine the effects of fungicide and variety on sporulation and inoculum density of *B. sorokiniana*. Soil was dry and had been since harvest. Samples were taken using a rectangular metal baking pan measuring 215 mm in length and 110 mm in width. This was pushed into the soil at a random position length-wise along a plant row (covering the plant crowns), to 6 cm depth (approximate sowing depth). Using a small mattock, soil was removed to form a trench on one side of the pan. The pan was tilted into the trench and the soil and plant matter it had enclosed was scooped up. Two samples were taken from each plot and pooled, giving a composite sample of 3 kg, representing 1.2% of the total row length. Samples were sieved through a 5 mm wire mesh. Plant trash was removed, weighed and the plant crowns were counted. The soil was well mixed and a sub-sample of about 300 g taken. Soil population densities of *B. sorokiniana* were determined (propagules per gram of soil), with two DR70 plates per plot, taken from the same soil dilution. Two further variates were calculated by dividing the inoculum density by the number of plants (crowns) and the weight of plant debris in the sample.

For both wheat and barley, the effects of the factors Variety and Fungicide rate on the variates were analysed. For variates measured at all sites, Site was included as a factor in the analysis.

8.3.6.2. Results and Discussion

1. Wheat

Sites differed in incidence and severity of common root rot on both wheat and barley (both variates $p < 0.001$) (Figure 8.14). Varieties of wheat were ranked consistently between sites for incidence and severity. The susceptible variety, Machete, had significantly higher disease severity (about 35%) than the two less susceptible varieties, Warigal and Aroona, although these latter two were differentiated by severity but not by incidence of disease symptoms (Figure 8.15).

Wheat varieties responded differently to fungicide rates, the Fungicide *Variety term being significant in the linear model for incidence ($p < 0.001$) (Figure 8.16) and severity ($p < 0.01$) (Figure 8.17) although all responses were slight only. With both variates, the interaction was due to a slightly greater response to fungicide rate in the susceptible variety, Machete (from the regression, about 29% reduction in severity at 200 g a.i./ha), than in the two less susceptible varieties (about 20% reductions).

There was no evidence of phytotoxicity and no effect on yield of wheat, negative or positive. Neither were expected, since crop stands did not visibly differ, and disease reduction by fungicide was small.

Plots of wheat at Mannum differed visibly in frequency of deadheads. This was not shown to be a result of fungicide treatment (n.s.), but was related to varieties ($p < 0.001$), as their different maturity dates would have given different premature ripening responses to moisture stress in spring. These data are not pertinent to this fungicide study and are not shown.

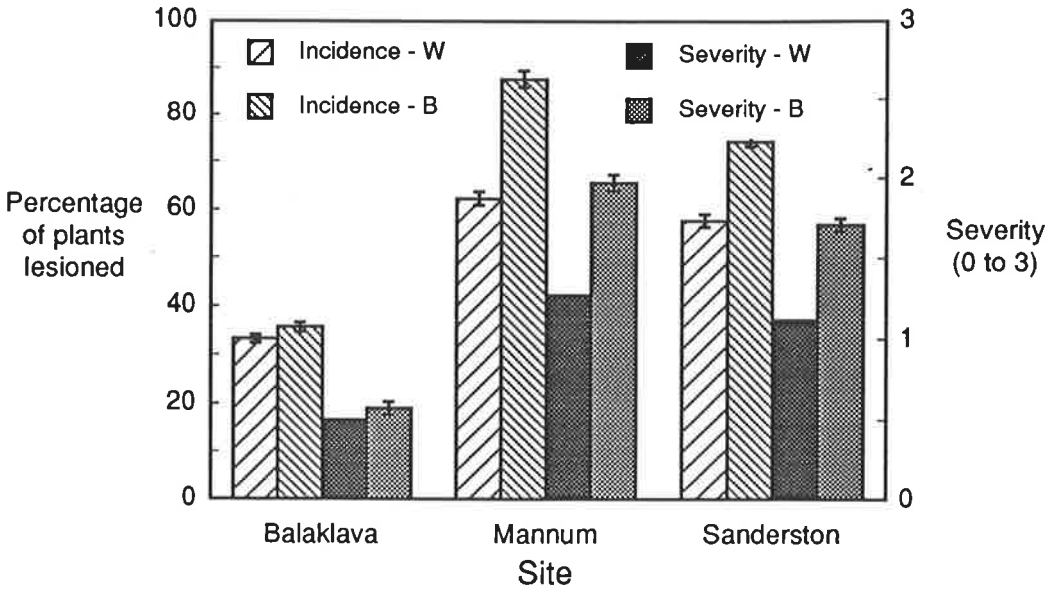


Figure 8.14. Incidence and severity of common root rot on wheat (W) and barley (B) at three sites in 1987. Error bars are standard errors of means, n = 105, for comparison only within crops.

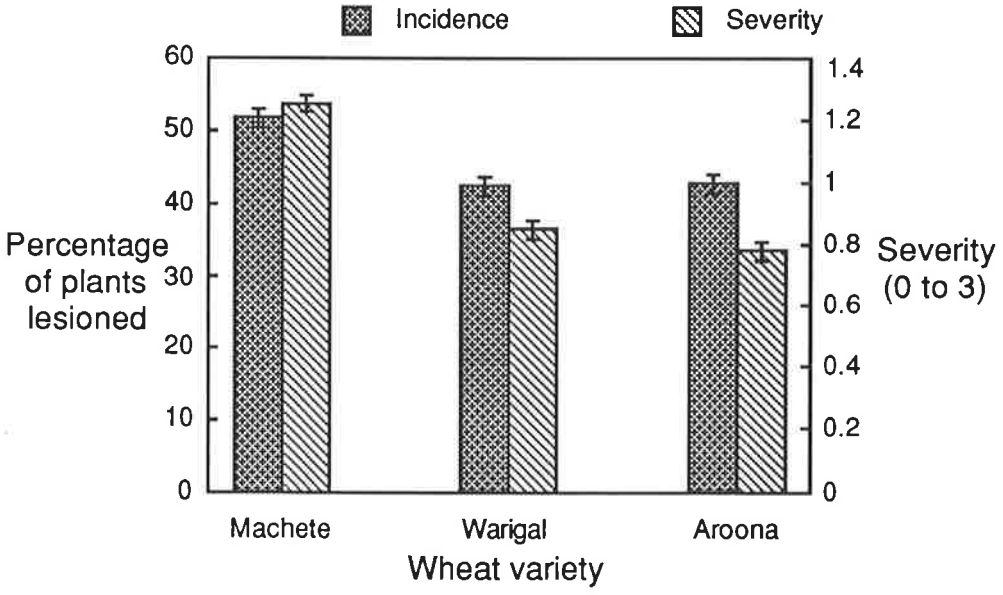


Figure 8.15. Incidence and severity of common root rot lesions on three wheat varieties, Machete, Warigal and Aroona at three sites in 1987. Error bars are standard errors of means, n = 315.

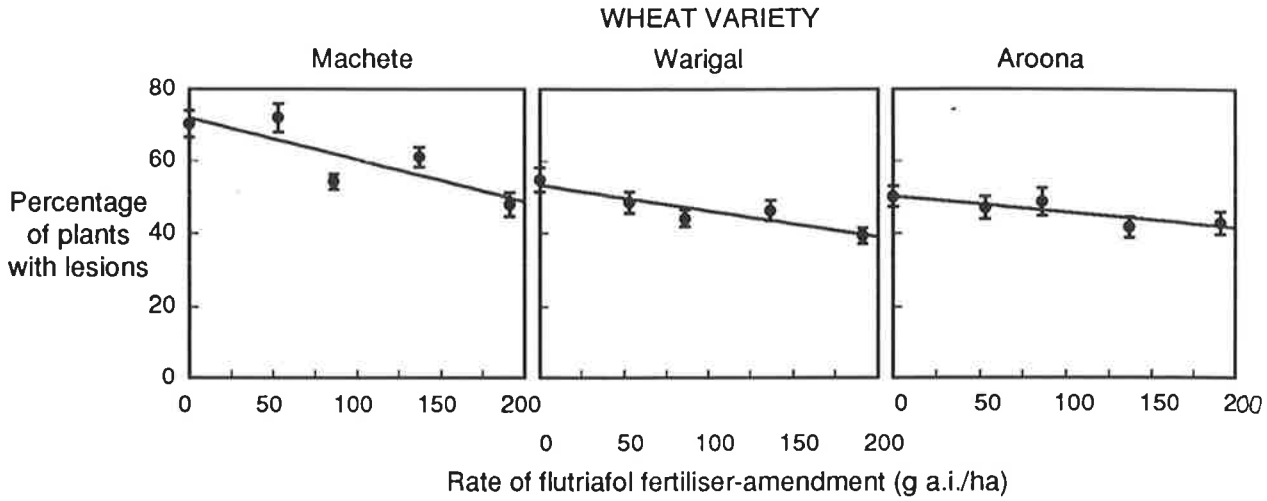


Figure 8.16. Effect on incidence of common root rot of flutriafol applied at five rates as fertiliser-amendment to three varieties of wheat at three sites in 1987. Error bars are standard errors of means, n = 21.

y (Machete) = 72.0 - 0.12x, r^2 = 0.70.

y (Warigal) = 53.2 - 0.071x, r^2 = 0.85.

y (Aroona) = 50.2 - 0.04x, r^2 = 0.77.

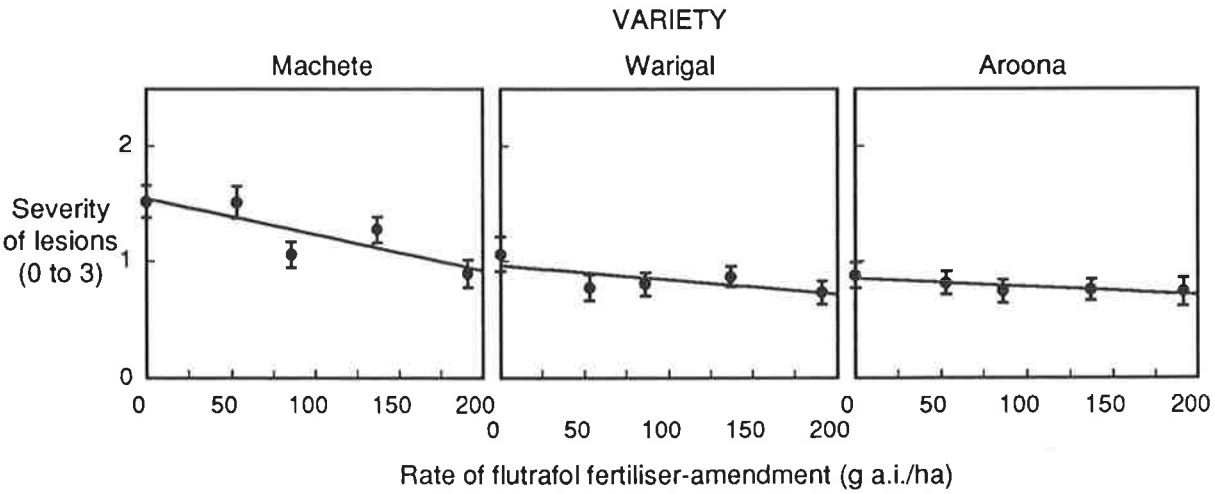


Figure 8.17. Effect on severity of common root rot of flutriafol applied at five rates as fertiliser-amendment to three varieties of wheat. Error bars are standard errors of means, n = 21.

y (Machete) = 1.53 - 0.003x, r^2 = 0.70.

y (Warigal) = 0.96 - 0.001x, r^2 = 0.50
y (Aroona) = 0.86 - 0.001x, r^2 = 0.04

2. Barley

In barley, common root rot incidence and severity were negatively associated with fungicide rate, but the responses varied between sites and were very small. The Fungicide*Site interactions were significant for incidence ($p < 0.01$) (Figure 8.18) and severity ($p < 0.001$) (Figure 8.19), associated with a lack of dose response at Sanderston Airstrip.

The interaction term of Site*Variety was significant for barley for incidence ($p < 0.001$) and severity ($p < 0.05$), due to the varieties differing in disease ratings only at the site with low disease levels, Balaklava (Figure 8.20). The differences were very small, although the higher disease levels in Forrest than Schooner and Galleon were consistent with their resistance rankings (Table 6.7). This result contrasts with the more substantial differences in resistance between wheat varieties, observed at all sites (Figure 8.15 and Chapter 6).

Fungicide had no significant effect on yield of barley.

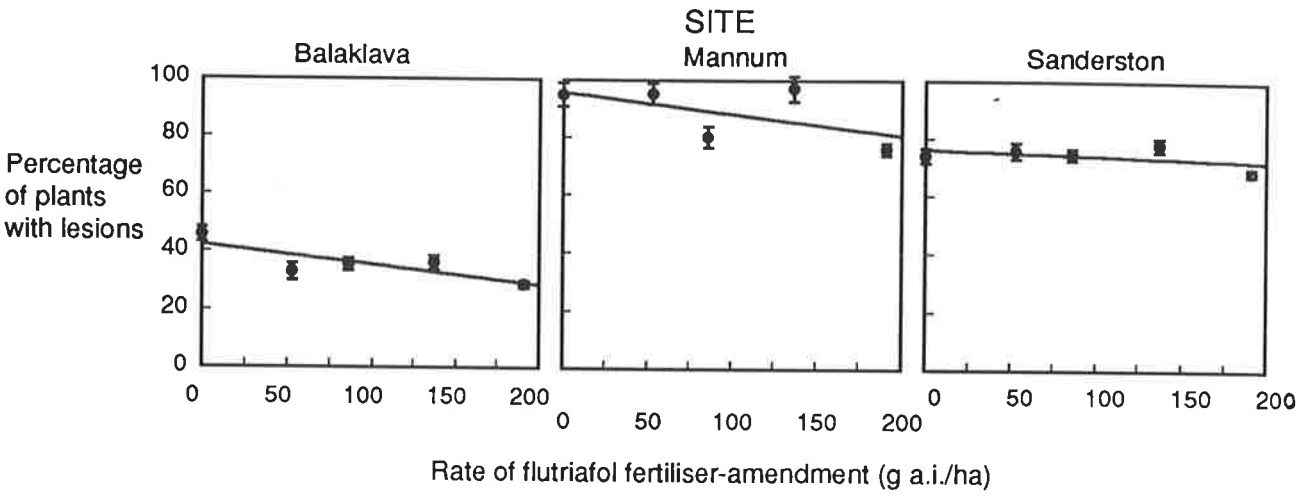


Figure 8.18. Effect on incidence of common root rot of flutriafol applied at five rates as fertiliser-amendment to barley at three sites in 1987. Error bars are standard errors, n = 21.

y (Balaklava) = 42.95 - 0.07x, r^2 = 0.64.

y (Mannum) = 95.39 - 0.095x, r^2 = 0.67.

y (Sanderston) = 76.08 - 0.021x, r^2 = 0.19.

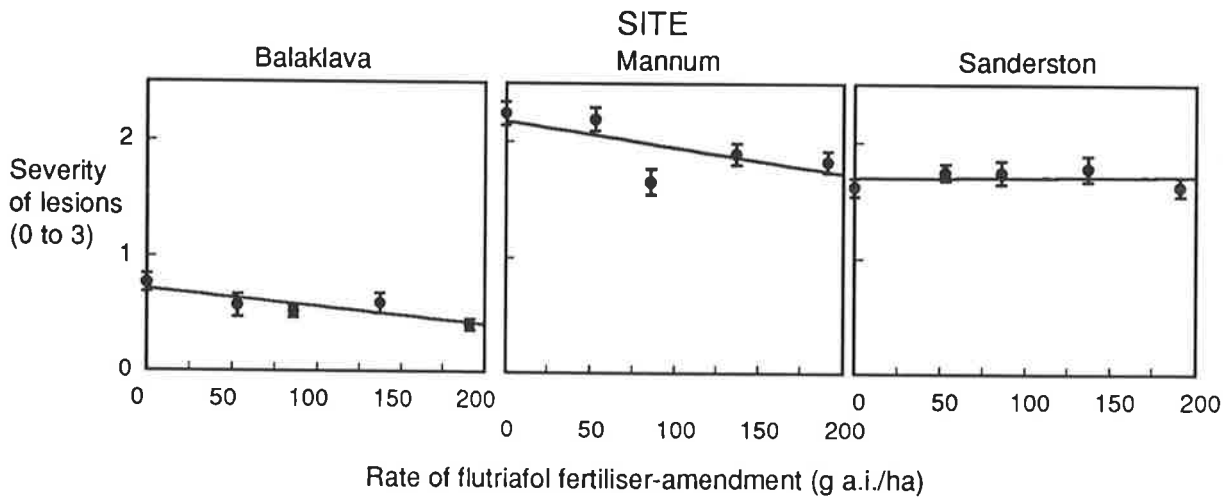


Figure 8.19. Effect on severity of common root rot of flutriafol applied at five rates of fertiliser-amendment to barley at three sites in 1987. Error bars are standard errors, n = 21.

y (Balaklava) = 0.71 - 0.002x, r^2 = 0.72.

y (Mannum) = 2.19 - 0.002x , r^2 = 0.46.

y (Sanderston) = 1.70, r^2 = 0.

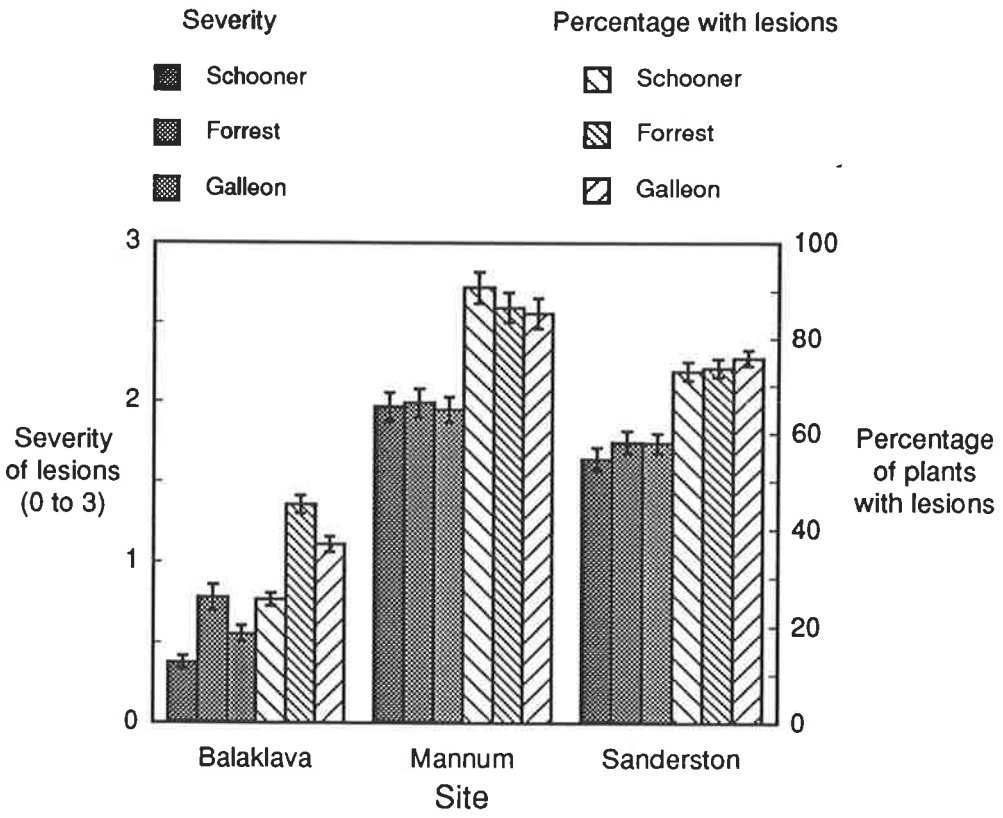


Figure 8.20. Incidence and severity of common root rot on three varieties of barley at three sites in 1987. Error bars are standard errors of means, n = 105.

3. Soil inoculum density of *B. sorokiniana*

Soil inoculum density of *B. sorokiniana* at Mannum was reduced by fungicide treatment in both wheat and barley at $p < 0.05$ (Figure 8.21). The dose responses of soil inoculum density were similar in pattern to those of incidence and severity (Figures 8.16, 8.17, 8.18 and 8.19). In wheat, the reduction in inoculum density by 50 g a.i./ha of fungicide was 24%, while in barley, it was 33%.

In both crops, soil inoculum densities in the 86 g a.i./ha treatment were lower than might be expected from the trends. No biological explanation for this is apparent. The independent analysis by gas chromatography precludes the explanation that fungicide application in this treatment was greater than the nominal rate.

Fungicide applied in the furrow could affect *B. sorokiniana* inoculum density in the soil by direct action on the fungus, or could act after uptake by the plant. Action of the fungicide must have been, at least substantially, to reduce sporulation on plants. This was demonstrated by the fact that dividing soil inoculum densities by plant density and plant biomass made no appreciable changes to the dose responses (Figure 8.21) (responses all significant at $p < 0.05$), that is, inoculum densities were proportional to plant density and biomass.

Reductions in soil inoculum density, while consistent in pattern with the dose responses of severity and incidence, were greater in proportion to scale. This may indicate that sporulation was not directly proportional to the amount of tissue infected. However, disease scoring was done near plant maturity. As demonstrated in Experiment 1, differences in common root rot can be greater earlier in the season, when fungicide activity is greater, and soil inoculum densities may reflect sporulation which occurred at that time.

Although wheat varieties differed in disease levels (Figure 8.15), this did not result in a significant effect on inoculum density, although the variety mean inoculum densities (103, 88 and 81 propagules per gram of soil form Machete, Warigal and Aroona respectively) were consistent with the variety mean disease levels (Figure 8.15) and with their resistance ratings (Table 6.3). The differences in disease levels between varieties were greater than those between fungicide rates. It is not clear, then, why fungicide treatment and not variety should have a substantial effect on inoculum density. This question, and the mode of action of the fungicide, could be investigated by repeating the experiment and taking smaller, more precise samples of soil and plant tissue for isolation of *B. sorokiniana* at several times through the growing season.

Inoculum density was 46% higher in wheat than in barley in the plots with no fungicide treatment. This was probably due to crop factors, rather than a block effect, because the two experiments were adjacent end-to-end, and a block effect would have

been evident in a trend across replicates, which was not the case. This is not consistent with the finding of higher incidence and severity on barley than on wheat (Figure 8.14), and the results of Chinn (1976b) that inoculum production was higher on barley than on wheat.

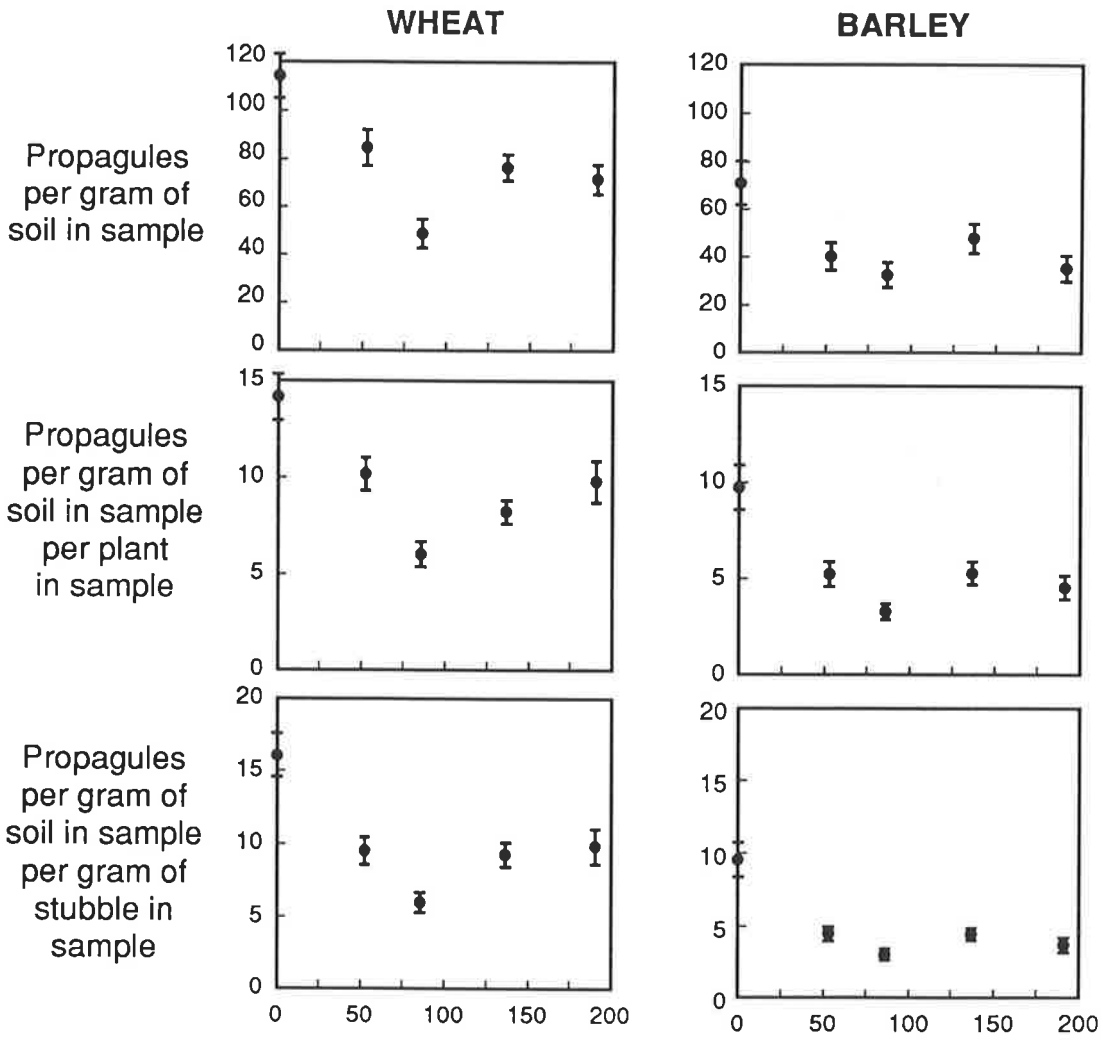


Figure 8.21. Effects on inoculum density of *B. sorokiniana* in the soil of flutriafol applied as fertiliser-amendment at five rates to wheat (left) and barley (right), at Mannum in 1987. Soil inoculum density (propagules/gram of soil) was divided by the number of plants (crowns) and the weight of stubble in 1.2 kg samples, to obtain the adjusted variates propagules/g/plant and propagules/g/g stubble. Error bars are standard errors of means, n = 21.

8.4. General discussion

The preliminary finding that flutriafol reduced common root rot on wheat was repeated in experiments with both methods of application, seed-dressing and fertiliser-amendment (Experiments 4 and 5). Application by seed-dressing was efficacious at 50 ppm, the concentration of the commercial formulation Vincit, and there was little further response at higher rates, including 100 ppm, the concentration of the commercial formulation Armour C. On the basis of disease control alone, then, the fungicide may be considered for commercial use for control of common root rot.

However, the experiments raised questions about whether the fungicide is safe for use on crops, because of its phytotoxicity, which precluded any yield responses which may have resulted from disease control. The most obvious effect on plant growth was stunting of the coleoptile (Experiment 2), resulting in delayed emergence and reduced plant biomass, tiller density and yield. This was also observed by Radford *et al.* (1989). The fungicide also affected flag leaf necrosis and deadhead incidence, probably by delaying maturity (Experiment 1). These effects tended to make the treated plots look more vigorous, but delayed maturity is detrimental to crops in much of South Australia, where moisture becomes limiting late in the growing season.

The significance of the coleoptile-shortening effect was probably exacerbated by sowing the seed deeply in order to enhance common root rot scoring. However, this does not invalidate the application of the adverse findings of phytotoxicity to commercial farming, since seed is frequently sown deep in crops due to either poor technology, or to variations in soil texture, which can cause variations in sowing depth even with good technology.

The dose responses of disease levels differed between the methods of application, seed-dressing and fertiliser-amendment, the first being cubic and the latter linear, although a direct comparison was not made. The linear reduction of disease observed in Experiment 5, up to the maximum dose of fungicide used (200 g a.i./ha), was less than that observed with the lowest dose of seed-dressing fungicide (50 ppm) in

Experiment 1. However, the magnitude of the response to the higher, single rate of flutriafol applied by fertiliser-amendment in Experiment 4 (243 g a.i./ha) was similar to that caused by 50 ppm of flutriafol seed-dressing in Experiment 1. Thus, the dose response in Experiment 5 may have represented the first portion of the cubic curve, so that greater rates than 200 g a.i./ha might not continue to give a linear response.

Phytotoxicity was not evident when flutriafol was applied by fertiliser-amendment, presumably because of much slower uptake of the fungicide. There was no significant effect on yield, although the small effect on disease levels observed would not be likely to lead to a yield response when possible yield losses from common root rot, judging from North American studies (Machacek, 1943; Ledingham *et al.*, 1973), may be 5 to 15%.

Consistent with the reduction in visual symptoms on the subcrown internode, both methods of fungicide application affected infection/inoculum of *B. sorokiniana*, by reducing its isolation frequency in Experiment 3 and its soil inoculum density in Experiment 5. Salas (1991) also found reduced isolation frequencies of *B. sorokiniana* on wheat grown from seed treated with fungicide (imazalil) and Verma *et al.* (1981) reported from preliminary studies that less sporulation occurred on tissues from fungicide-treated plants than from untreated ones. Thus, the fungicide may also reduce disease levels in following crops, providing that inoculum is reduced to a density lower than that which could cause maximum disease levels in the environmental conditions prevailing in the following season (see Section 2.10).

Stack (1991) questioned the validity of the subcrown internode disease index for comparison of fungicidal seed-dressings, because the disease affects the roots also (Simmonds *et al.*, 1935; see Section 5.2.3) and the fungicides may not affect the disease on the roots. However, this is the case for most fungicides applied by seed-dressing (Scheinpflug and Duben, 1988), so the index may be generally valid for comparing fungicides. The main problem with the index in this application is the difficulty of scoring distorted internodes, as experienced in Experiment 3. Possibly,

isolation frequency of *B. sorokiniana* from the crown or the subcrown internode (distorted or otherwise), is an acceptable alternative method. The water agar/DR70 sandwich method (see Section 5.2.3) would be valuable for this purpose.

Triadimefon, which reduced common root rot in experiments in the USA (Stack, 1991) and the related compound triadimenol, which is active against *B. sorokiniana* (Scheinpflug and Duben, 1988), had no significant effect on common root rot. This was supported by it being no different to the control in isolation frequency of *B. sorokiniana* from subcrown internodes. The discrepancy may be due to formulation; a dry powder was used in the experiments reported here, whereas the USA formulation is a slurry and wet formulations are more active (Scheinpflug and Duben, 1988).

The experiments demonstrated a fundamental flaw in the use of fungicides for evaluating yield losses from disease. Not only did the fungicides directly affect plant growth (and this may be positive or negative), but also, although the results are not reported here, they concurrently affected levels of take-all (*G. graminis* var. *tritici*) in wheat and powdery mildew (*Erisyphe graminis*) in barley. These effects can be partitioned using covariate analysis, providing they are closely observed and measured, but often they may not be observed or measured and will be confounded.

The study was enhanced by the use of assay procedures for flutriafol. In an experiment not reported here, for which pretreated fertiliser was used, consistent departures from linearity of responses indicated that actual rates were different to nominal rates. In Experiment 5, in which fertiliser was amended with all due care and then assayed, non-linearity still occured, indicating that errors were present in the method. The fertiliser was highly heterogeneous in particle size, and this would lead to problems with consistency of adherence of fungicide to the fertiliser particles or with evenness of application. This is an inherent barrier to performing critical experiments with this method of fungicide application.

(no page 175)

9. SUMMARY

Prior to Tinline's (1984) study, there was little awareness of common root rot in South Australia, although an early indication of its potential importance was given by Samuel's (1924) finding, that 64% of plants in a crop of wheat near Pinnaroo were infected with *B. sorokiniana*.

The survey by Tinline (1984), in three geographically diverse regions of the state, showed that *B. sorokiniana* was common in soils, at inoculum densities comparable to those in Canada (Duczek, 1981) and Queensland (Wildermuth, 1986), and that common root rot affected more than 80% of plants on average. Fedel-Moen and Harris (1987) observed high rates of infection of barley by *B. sorokiniana,* but found several *Fusarium* spp. also to be associated with root rotting. This study led them to propose that common root rot in South Australia was caused by a complex of fungi. Lewis (personal communication) conducted preliminary studies of resistance to common root rot in wheat, and found that genotypes varied in their disease ratings, and that moderately resistant genotypes were frequent. Rathjen and Pederson (1986) suggested that, as the wheat breeding method used in the state can unwittingly select for characters which improve yield, a high frequency of genotypes resistant to a disease indicates that the disease had been affecting yields.

Given that common root rot causes yield losses in other areas of about 5 to 15% (Machacek, 1943; Ledingham *et al.*, 1973; Piening *et al.*, 1976), these South Australian studies indicated that the disease may be far more important than previously recognised, and further studies were justified. The studies reported in this thesis were intended to improve understanding of the disease in South Australia.

Investigations

A survey of common root rot was conducted in wheat and barley crops across the state. Disease incidence was mapped , and levels of incidence and severity were

compared between regions and years. The rate of development of common root rot through the season was observed at six sites.

Investigations using Koch's Postulates were undertaken on the role of *B. sorokiniana* in causation of common root rot. Isolation frequency of the fungus was determined in samples from 115 wheat crops from seven regions of the state. Subcrown internodes with and without lesions were plated and the isolation frequencies of *B. sorokiniana* were compared. Isolates of *B. sorokiniana* from both wheat and barley were inoculated onto wheat and barley, in a controlled environment which was intended to mimic field conditions in respects important to the biology of the disease. Disease severity and isolation frequency of *B. sorokiniana* were compared between isolates and hosts. The association between disease severity, and the isolation frequency and vigour of *B. sorokiniana* were examined.

Field experiments were conducted to examine the range of resistance to common root rot among both wheat and barley. The consistency of responses between sites was evaluated.

The effects of three important agronomic factors on soilborne inoculum of *B. sorokiniana* were examined over two years, in a long-term field trial incorporating, as factors, five crop rotations, two intensities of tillage and two soil types.

Fungicides which are used in South Australia for control of head and foliar diseases in wheat and barley were tested for their effects on common root rot incidence and severity, plant growth and crop yield, and the rate of infection and soil inoculum density of *B. sorokiniana*.

Results

Common root rot was present throughout the state's cereal belt in many different environments. In 1988, the mean incidence of diseased plants in crops was 60% in wheat, and 77% in barley. The corresponding values in 1989 were 34% and 49%. Temperature during winter was probably an important determinant of annual variation

in disease levels. Regions varied in mean disease levels, and soil types, rotations and temperature may have contributed significantly to these differences. Levels of common root rot in wheat crops corresponded with their varietal resistance ratings, with significantly less disease on moderately resistant varieties than on others.

Progress of common root rot through the growing season in Machete wheat varied between six field sites in 1987. Percentage of plants with lesions ranged from 0 to 60% 83 days after sowing, and from 37 to 95% after 175 days. The differences between sites indicated that factors in addition to temperature were important in determining disease development rate and final levels.

B. sorokiniana, the putative common root rot pathogen, was isolated from 105 out of 115 wheat crops in 1988, with a mean isolation frequency of 42% from lesioned subcrown internodes. In Machete wheat in the field in 1989, *B. sorokiniana* was isolated from 9% of unlesioned subcrown internodes and 43% of diseased ones. In the pathogenicity test, lesions on the subcrown internode and roots, such as observed on field samples, were present in both inoculated and control treatments, but were more severe in the inoculated. *B. sorokiniana* was more frequently isolated from inoculated than control plants. There was a strong correlation ($r = 0.81$) between lesion severity and isolation frequency. Slightly diseased tissue gave rise to smaller colonies than did severely diseased tissue. In this work, a plating method ("water agar/DR70 sandwich") was developed for selective isolation of *B. sorokiniana*, using the selective medium of Dodman and Reinke (1982). The sandwich gave improved selectivity, was highly efficient, allowed accurate quantitation of disease severity and tissue colonisation, and allowed the association of these two variates to be investigated.

In both wheat and barley, there was variability in disease severity among genotypes, and ratings were quite consistent between sites, although the range and consistency was much greater in wheat than in barley. Genotype*site interactions were observed, but may have been statistical artefacts, rather than true interactions. Wheat varieties

covering a large proportion of the state's wheat area were rated moderately susceptible and susceptible. Others had much lower disease levels and were rated moderately resistant. These would be useful as resistance sources for breeding, without resorting to donors unadapted to South Australian conditions. Field screening could be used for resistance breeding in wheat. Without better resistance than available locally in barley, breeding for resistance using field screening would be difficult in that crop.

Flutriafol reduced symptoms when applied by seed-dressing or fertilser-amendment, in contrast to triadimefon, which had no effect. A 25% reduction in disease incidence at anthesis and maturity resulted from flutriafol at low rates (50 parts per million of seed-dressing or 50 grams of active ingredient per hectare of fertiliser-amendment). At higher rates, about 50% reduction was observed. The fungicides were phytotoxic, causing reduction in coleoptile length, delayed emergence or reduced establishment, thickened and shortened subcrown internodes and delayed maturity. This was exacerbated by deep sowing, done deliberately to induce formation of long subcrown internodes for scoring common root rot. Mostly, yields were not affected, but were reduced in one case. Flutriafol seed-dressing reduced the frequency of isolation of *B. sorokiniana* from subcrown internodes of barley, while triadimefon did not. Applied as a fertiliser-amendment, flutriafol reduced the population density of *B. sorokiniana* in the soil by about 55%, to about 60 ppg in barley and 40 ppg in wheat.

Among the five crop rotations studied, the highest mean inoculum density was after continuous wheat, with 168 propagules per gram of soil (ppg). Peas and oats in the rotation resulted in non-significant reductions to 146 and 137 ppg respectively. Population densities after grassy pasture and medic were 108 and 104 ppg, both significantly lower than in continuous wheat. Population density was significantly lower with direct drilling than with conventional cultivation (109 versus 144 ppg). Sandy loam had a significantly higher population density than clay loam (147 vs. 117 ppg). None of the treatments reduced inoculum sufficiently to greatly reduce

common root rot levels, although they could be useful in an integrated control program.

Conclusions

The very wide distribution of common root rot and its cause, *B. sorokiniana*, demonstrate that it is potentially an important determinant of crop yields in South Australia. The wide range of environments in which the disease occurred shows that the findings of these studies can be extrapolated reliably to the winter rainfall-dominant areas of Victoria, New South Wales and Western Australia. Environmental conditions which were associated with high disease incidence and severity are reasonably common, and only a small proportion of the wheat and barley crops are protected by resistance.

It is unsafe to assume that yield losses occur in South Australia to the same extent as estimated in North America (5 to 15%), because of the large difference in length of growing season (170 vs. 100 days respectively) and the different rates of disease development. However, the wide distribution of the disease would magnify even small losses in individual crops, to large economic losses over the state.

Preliminary studies of yield losses from common root rot in wheat and barley in South Australia reported by Whittle *et al.* (1991), indicated that yield losses of up to 30% had occurred in individual crops, but there were ambiguities in the results, possibly due to methodology. Because of the high risk of losses, these studies should be continued, so that the economic importance of common root rot in South Australia, and regions climatically and edaphically similar, is resolved.

The studies provided strong evidence that *B. sorokiniana* was responsible for the majority, if not all, of the common root rot symptoms observed. No justification was found to adopt the hypotheses that the disease was caused by a complex of fungi, or by a fungus other than *B. sorokiniana*. Consequently, control methods found to be effective against common root rot could be confidently promulgated.

In wheat, the risk from common root rot is greatest in areas with sandy soils, and/or rainfall too low to permit the use of legume crops in rotation. The use of resistant varieties and, depending on practical considerations, direct drilling, are advisable in these areas. Flutriafol should be preferred over triadimenol for seed-dressings in these areas. It is warranted to select for resistance to common root rot, when breeding wheat adapted to the drier environments.

Resistance and choice of fungicide are less important in wheat where sown legume crops and pastures are grown, since less disease was found in these areas, and these species can reduce inoculum levels. However, since barley is sown after wheat, which in one crop can increase inoculum density to previous levels (Wildermuth and MacNamara, 1991), it may be important to prefer a flutriafol-based seed dressing on barley in these regions also. Given the importance of rotations in minimising cereal cyst nematode (*H. avenae*) and take-all (*G. graminis* var. *tritici*) (Taylor, 1991), it is not warranted to structure crop rotations specifically to control common root rot.

Resistance of wheat is possibly being improved in the existing breeding programs, and moderately resistant genotypes are common among parents and progeny. Intensity of selection could be increased, by locating yield trials where common root rot is likely to occur (e.g. sandy soils, high density of *B. sorokiniana,* severe disease previously observed), or establishing nurseries by choice of crop rotation, or by growing plants in the glasshouse in soil infested with *B. sorokiniana.* It would certainly be warranted, in the Roseworthy program, to select within the RAC177 family for high-yielding, moderately resistant lines which could be used for parents, in preference to the highly susceptible genotypes which predominate in this family.

There is little prospect for improvement of the resistance of barley through breeding, because there is a paucity of effective resistance, the small differences are difficult to select for, and they do not relate proportionally to yield losses (Tinline and Ledingham, 1979). Analysis of the literature, and results of the field studies, show that a greenhouse test, in which conditions are manipulated to slow disease

development through the seedling stages, may allow better and faster evaluation of resistance than in the field. Given the high disease incidence and severity observed in barley, it is warranted to study this further.

Results of the study on soil inoculum density of *B. sorokiniana* in wheat and barley after application of flutriafol by fertiliser-amendment are grounds for further studies, although these treatments are unlikely to be commercialised. Wheat varieties differing in resistance to common root rot did not vary significantly in soil inoculum density of *B. sorokiniana,* while a reduction in disease levels by fungicide, comparable in magnitude to the effect of resistance, led to a substantial reduction in inoculum density. The reason for this was not clear, and might be revealed by a detailed study of population dynamics of *B. sorokiniana* through the growing season, with fungicide treatment and different varieties of wheat. Barley should be included, since it had lower inoculum density than would be expected from its disease severity, in comparison with wheat.

There was a lack of information on the effects of water energy and availability on common root rot, although the studies of McKinney (1923), Bailey *et al.* (1989) and Duczek (1990), demonstrate this to be an important issue. Further studies should be undertaken to clarify the effects of water potential on the biology of *B. sorokiniana* and common root rot. This work is important to achieve better repeatability in research, to more easily predict the effects of seasonal climatic conditions on the disease, and to explain the effect of soil types on inoculum of *B. sorokiniana* and levels of the disease. The method used to control water potential in the pathogenicity test may be useful for these studies, and for similar work on other host-pathogen systems.

APPENDICES

Appendix 1. List of towns and places in South Australia mentioned in text, with map references. These can be located on the map in Figure 1.1.

Town/Place	Latitude (S)	Longitude (E)
Adelaide	138°36'	34°55'
Avon	138°20'	34°17'
Balaklava	138°26'	34°7'
Ceduna	133°40'	32°8'
Cleve	136°30'	33°42'
Coonalpyn	139°51'	35°23'
Cungena	134°42'	32°13'
Darke Peak	136°8'	33°30'
Geranium	140°10'	35°23'
Kadina	137°40'	33°57'
Kapunda	138°55'	34°20'
Lameroo	140°31'	35°20'
Long Plains	138°23'	34°21'
Loxton	140°35'	34°27'
Maitland	137°40'	34°22'
Mallala	138°30'	34°26'
Mangalo	136°30'	33°32'
Mannum	139°18'	34°55'
Mudamuckla	134°3'	32°13'
Nangari	140°53'	34°29'
Pinnaroo	140°55'	35°16'
Roseworthy	138°45'	34°34'
Sanderston	139°13'	34°46'
Sedan	139°16'	34°35'
Streaky Bay	134°12'	32°50'
Tepko	139°16'	34°57'
Windsor	138°20'	34°25'

Appendix 2. Method for determination of flutriafol concentration in fungicide-amended superphosphate fertiliser.

Method used by G. Gould (Victorian Department of Agriculture and Rural Affairs, Victorian Crops Research Institute, PB 260, Horsham, VIC 3400) for analysis of samples for Experiment 4 (Chapter 8).

A 100 g sample of superphosphate fertiliser was ground using a Bamix mill. A 20 g subsample of the ground superphosphate and 1 g of Ca(OH) were placed in a 250 ml conical flask and mixed well. Thirty ml of distilled water was added and stirred to dissolve the superphosphate. Fifty ml of nanograde dichloromethane was added and the slurry was shaken on an orbital shaker for 30 minutes.

The sample was filtered using a sintered glass funnel, then re-extracted with a further 30 ml of dichloromethane and 20 ml of distilled water. The internal standard was added at this stage and the sample was shaken for 15 minutes. Filtering was repeated and the two filtrates combined.

The dichloromethane was evaporated, and the extract was dissolved in ethyl acetate. Flutriafol concentration was determined by gas chromatography.

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