



**Nutritional requirement of wheat in relation to
tolerance to *Rhizoctonia solani* Kühn**

by

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This thesis is presented for the degree of

Doctor of Philosophy

in

*The department of Plant Science
Waite Agricultural Research Institute
The University of Adelaide*

February 2000

Declaration

I declare that this thesis is my account of my own work, none of which has been submitted previously in fulfilment of requirements for a degree at any University and, to the best of my knowledge, contains no material previously published or written by another person, except where due reference has been made in the text.

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Julie A. Cooke

Acknowledgements

I would like to acknowledge the help of my supervisors, Professor Robin Graham, Dr. Zed Rengel and Dr. Nick Pearson who provided constructive criticism of the thesis and research. I would like to thank all the members of the Trace Element group at the Waite Institute for their support and patience. Without the support of family and friends I don't know how I would have completed writing. Last but not least I thank my husband Steve who was a fantastic help during the time it took to complete this thesis.

Abstract

Rhizoctonia solani Kühn is one of the major root rot pathogens in the Australian cereal belt. *Rhizoctonia* infection or root rot is responsible for significant losses in yield. At present there is no biological control method for the suppression of *R. solani* infection in wheat, nor has a resistant cultivar been identified.

It has previously been demonstrated that zinc-deficient plants are more susceptible to *R. solani* infection than counterparts supplied with adequate nutrients. Here the possible advantages in the application of zinc fertiliser for the reduction in the incidence of infection has been investigated. Furthermore, a variety of wheat cultivars were screened to determine variability in tolerance to the fungus.

Preliminary experiments were conducted to allow for a system of experimentation be established. To determine the effect of Zn source on the growth of wheat infected by *R. solani* a factorial experiment with two levels of applied Zn (0 and 0.4 mg Zn kg⁻¹), two levels of seed Zn content (low and high), two levels of *R. solani* inoculum (0 and 16 propagules kg⁻¹ soil), and two cultivars (Excalibur and Gatcher) was performed. This experiment demonstrated that plants infected by *R. solani* had improved vegetative yield when supplied with Zn, either in the seed or by the application of Zn to the soil. Furthermore this experiment demonstrated that Excalibur displayed a lower percentage reduction of dry matter when infected with *R. solani* regardless of Zn application or seed Zn content compared to Gatcher. Zinc application, to a zinc-deficient soil, did not reduce the severity of *R. solani* infection but the application of zinc did decrease the amount of yield lost as a consequence of the infection.

To determine if Zn was unique in improving yield of wheat infected by *R. solani* or if the addition of P also improves yield in *R. solani* infected plants when plants are grown at a

limiting P supply two subsequent factorial experiments were conducted. Plants responded similarly to the stress imposed by either P or Zn deficiency or *R. solani* infection. *Rhizoctonia solani* inoculation of Songlen wheat resulted in increased root length, in both the Zn and P experiments, an increase in the number of laterals per unit length of seminal root and an increase in the mean length of 1st-order laterals. Comparing results of Zn and P experiments it was concluded that adequately nourished (Zn or P) Songlen wheat responded to *R. solani* infection by altering root morphology, such as increasing lateral root production. The decrease in yield loss owing to infection by *R. solani* when plants were supplied with adequate zinc may be due to a better ability of the plant to take up zinc.

To further understand the effect of Zn supply on seedling susceptibility to *R. solani* infection a factorial experiment was conducted with four levels of added Zn (0, 0.0-5, 0.2 and 2 mg Zn kg⁻¹), two inoculum densities (0 and 16 propagules) and three wheat cultivars (Songlen, Janz and RAC 655). Root morphology between control and infected plants was investigated. As the fungal pathogen macerates the root system of the host plant, it was hypothesised that the loss of functional roots would reduce the capacity for adequate water and nutrient uptake by the plant, and thereby reduce leaf growth.

The final experiment was designed to determine if Zn transport differs in cultivars of wheat when infected with *R. solani*. A factorial experiment combining three rates of soil-applied Zn (0, 0.04 and 0.8 mg kg⁻¹ Zn), two levels of *R. solani* inoculation (0 and 16 propagules) and three cultivars (Excalibur, Songlen and Gatcher) was conducted. The present research supports a hypothesis where the ability of a plant to take up, transport and utilise zinc whilst infected with *R. solani* is the basis for tolerance. The data suggest that cultivars of

wheat that have a high zinc transport efficiency are able to tolerate the disease to a greater extent than cultivars with a low zinc transport efficiency.

The research described here should provide the basis for a more detailed analysis of cultivar tolerance to *R. solani* in *Triticum aestivum*. Without *Rhizoctonia*-resistant cultivars, tolerance may provide the only real cost-effective method for controlling the disease.

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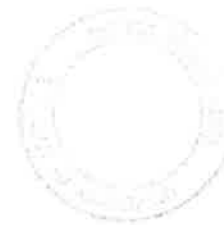
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Chapter 1

General Introduction

Australian soils are highly weathered and strongly leached, and therefore have low fertility (Taylor, 1983). Strategies to grow plants successfully in these soils are important in Australian agriculture. Agronomists must understand why closely related plant species or cultivars display different growth abilities under particular soil, water and nutrient regimes. Greater understanding of the relationship between plant cultivars and their ability to grow under given nutrient conditions allows optimisation of the plant nutrient status either by modifying the nutrient availability in the surrounding environment or modifying the ability of the plant to utilise nutrients already available.

Modification of nutrient availability in the plants' immediate surroundings through the application of fertilisers is the most common method of increasing the availability of limiting elements to the plant. However, the financial and environmental costs associated with fertiliser applications are making it increasingly necessary to explore alternative strategies for maintaining plant nutritional status. Nutrient uptake by the plant can be increased by genetically improving efficiency of the plant to absorb nutrients from the surrounding environment, translocate the nutrients within the plant, and to utilise nutrients in metabolic reactions (Graham, 1988; Huber and Wilhelm, 1988; Huber and Graham, 1998).

One of the major factors which may influence the growth of different plant cultivars under nutrient deficiencies has been the effect nutrient deficiency can have on the resistance or tolerance of the plant toward a range of diseases (Huber, 1981; Graham, 1983; Sparrow and Graham, 1988; Wilhelm *et al.*, 1988; Graham and Webb, 1991). Plants displaying nutrient deficiencies have been shown to be more susceptible

to a range of diseases, many of fungal origin (Graham, 1983; Graham and Rovira, 1984; Rengel *et al.*, 1994; Grewal *et al.*, 1996). It is not surprising therefore that more effort is currently being focussed on identifying cultivars displaying resistance or increased tolerance to pathogens under nutrient-deficient conditions. The incorporation of such cultivars into breeding programs can be expected to allow a reduction in the application of external nutrients required for plant growth in nutrient poor, pathogen-containing soils.

The terms resistance and tolerance applied to the host in a host-pathogen system relate to the growth of the pathogen and response of the host, respectively (Graham and Webb, 1991). Resistance of a host plant is measured in terms of its ability to inhibit (partially or fully) the development and reproduction of the invading pathogen (Graham and Webb, 1991). Tolerance of the host plant is measured in terms of its ability to maintain its own growth or yield in spite of pathogen infection (Trudgill, 1986). Wallace (1987) has argued that tolerance to pathogens is not a response specific to an invading pathogen, but is a normal homeostatic response of the host that has evolved to counter a wide range of environmental stresses, mostly abiotic. *Rhizoctonia solani* reduces the nutrient absorbing surface of the plant (by severing roots), hence if the plant is tolerant to nutrient-deficient soil, the plant may also be regarded as displaying a conferred tolerance to the pathogen. It is difficult in such cases to separate between different environmental stresses as one strongly affects the other. Although the physiological role of nutrients in resistance to disease is often obscure, their involvement as integral components of a cell's biochemistry (as substrates, enzymes and electron carriers, or as activators, inhibitors and regulators of metabolism) involves them in all potential aspects of pathogenesis (Huber, 1981).

Rhizoctonia root rot of wheat is caused by the fungus *Rhizoctonia solani* Kühn AG-8 and is responsible for causing "bare patch" in fields associated with substantial economic losses. The term "bare patch" arises because plants affected by this disease are severely stunted and occur in distinct patches (Weller *et al.*, 1986). *Rhizoctonia* is

found traditionally on sandy, calcareous soils in South Australia. Genetic resistance to *R. solani* has been reported in a number of non-cereal crops, such as lima bean (Steinswat *et al.*, 1967), snap bean (Dickson and Boettger, 1977), alfalfa (lucerne) (Barnes *et al.*, 1980) and pea (Shehata *et al.*, 1981) and has been demonstrated to be a heritable characteristic (Williams and Walker, 1966; Steinswat *et al.*, 1967; Dickson and Boettger, 1977). However, there is only one report of cereal resistance to root rot caused by *R. solani* (McDonald and Rovira, 1985). Neate (1989) has demonstrated small differences in resistance to *R. solani* between different cereal species, such as wheat, barley, rye and triticale, and among cultivars within these species. However, the variability found among the cereal cultivars tested was not large enough to warrant further research. It is plausible, however, that the small differences between cereal cultivars observed by Neate (1989) might become significant under nutrient-limiting conditions. The application of nutrients to *R. solani*-infected plants has been shown to reduce the symptoms of bare patch (Millikan, 1938). The application of nitrogen (N) (as ammonium sulfate, urea or sodium nitrate) has been associated with reduced *Rhizoctonia* bare patch or root rot (Hynes, 1937; MacNish, 1985). Wall *et al.* (1994) have demonstrated that the critical level of tissue N for optimum growth of wheat is the same in diseased and healthy plants but that there is a decreased growth response to added N in diseased plants. This suggests that diseased plants have reduced efficiency in N uptake, N translocation or N metabolism compared to healthy plants and is reflected in the observation that, in some situations, applications of N have failed to reduce *R. solani* infection (MacNish, 1988).

The beneficial effects of the application of Zn for the control of fungal diseases has been reported on several occasions (Millikan, 1938; Bolle-Jones and Hilton, 1956; Basu-Chaudhry, 1967; Sparrow and Graham, 1988; Thongbai *et al.*, 1993a). Thongbai *et al.* (1993 a, b) demonstrated that in low-zinc soils, applications of zinc reduced bare patch and root rot, but applications of Zn to Zn-sufficient plants did not further reduce disease. Grewal *et al.* (1996) further demonstrated wheat cultivars more efficient in

extraction of zinc from low-zinc soils are more resistant to infection by *Fusarium graminearum* than cultivars that display inefficient extraction of Zn from low-Zn soils. Cultivar variation in Zn efficiency and resistance to bare patch disease (*Rhizoctonia solani*) in wheat has not been previously demonstrated.

Plant breeding is an important procedure in the production of high-yielding cultivars that are resistant to, or tolerant of, a specific biotic or abiotic stress. The specificity underlying the determination of resistance or susceptibility to pathogens operates, in many cases, at the cultivar level. Cultivars exhibit differential responses to distinct physiological races of the pathogen (Lamb *et al.*, 1989). Knowledge of the resistance displayed by plant cultivars to pathogens has resulted primarily from research associated with the breeding of crop species by direct gene transfer methods (Darrah, 1993). It is known that plant genomes contain a large number of genes that are potentially involved in the detection and discrimination of pathogens (Crute and Pink, 1996). The problem in breeding for resistance lies in deciding which property to select for among a multiplicity of possibilities. Although disease resistance is genetically controlled, it is mediated through physiological and biochemical processes interrelated with the nutritional status of the plant or pathogen (Huber and Graham, 1998).

The breeding of plants to adapt to or to tolerate mineral deficiencies offers opportunities for increasing plant productivity (Clark and Brown, 1980). Plant breeders have been able to select cultivars of wheat and barley which are nutrient-efficient (the term “nutrient-efficient” applies to a cultivar or phenotype that is better adapted to, or can yield more in a deficient soil compared to the average of the species (Graham, 1991)). Nutrient-efficient cultivars may prove to be resistant to many diseases in nutrient-deficient soils because, compared with nutrient-inefficient cultivars, they may contain higher nutrient concentrations owing to a greater ability to extract and utilise limiting nutrients (Graham *et al.*, 1992).

The focus of the research contained in this thesis is on plant nutrition and its role in disease resistance of Australian wheat cultivars to the soil-borne root-rotting

fungus *Rhizoctonia solani* Kühn. The aim of the project is to determine whether cultivars of wheat, grown under different nutrient regimes display a difference in tolerance to *Rhizoctonia solani* and to try to decipher some of the underlying mechanisms.

Chapter 2

Literature review

2.1 Introduction

The beneficial effects of the application of nutrients for the control of fungal diseases has been reported on several occasions (Millikan, 1938; Bolle-Jones and Hilton, 1956; Basu-Chaudhry, 1967; Sparrow and Graham, 1988; Thongbai *et al.*, 1993a). In order to explore the relationship between the nutrient status of wheat and tolerance to infection by *R. solani*, it is first necessary to understand the related background. Consequently, the following review outlines the uptake and utilisation of Zn and P in wheat, describes the causative organism of *Rhizoctonia* bare patch, *Rhizoctonia solani* Kühn (AG 8), and gives an overview of the current status of research into the resistance of wheat to *R. solani*.

2.1.1 Importance of zinc

The first biological investigations of Zn nutrition describe its effect on the bread mould *Aspergillus niger*. In 1869, Raulin discovered that *Aspergillus niger* failed to undergo cell division when grown on Zn-deficient media (Underwood, 1971). However, it was much later that Zn was reported to be essential for higher plants (Sommer and Lipman, 1926). In the 1930s, Zn deficiencies in peaches, citrus and field crops were first identified (Chapman, 1966).

2.1.2 Elemental zinc

The requisite criterion for a transition metal is to have filled 4d orbitals (Barak and Helmke, 1993). Molybdenum (Mo) is the only true transition element with filled 4d

orbitals. The elements zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu), whilst in the same group as Mo, can not be regarded as true transition metals as they only have filled $3d$ orbitals (Barak and Helmke, 1993). Zinc has an outer pair of s electrons, and only these are involved in chemical reactions during which both electrons may be lost, such that Zn occurs in compounds only in the +2 oxidation state (Brady and Holum, 1988).

2.1.3 Zinc in soils

Zinc, by virtue of its low concentration in soils, is termed a trace element. Surveys have shown that the total Zn concentration in various Australian soils is generally in the range of 10-300 mg kg⁻¹ (Swaine, 1955; Jensen and Lamm, 1959), with the average for the lithosphere being approximately 80 mg kg⁻¹ (Goldschmidt, 1954). The total amount of Zn within a particular soil is dependent upon the soil type.

2.1.4 Plant-available zinc in soils

Plant roots generally access Zn from moist surface soil, and consequently current fertilisation practices in South Australia centre upon applying nutrients only to the surface soil. However, severe erosion of top soil may expose Zn-deficient subsoil increasing the likelihood of Zn deficiency in crops (Grunes *et al.*, 1961). Owing to severe erosion, many Australian farmers now apply conservation tillage or minimum tillage practices in an attempt to reduce soil loss. This technique therefore also minimises the loss of plant-available Zn by limiting top soil erosion.

In many South Australian soils, Zn is inadequately available to crops, despite being present in an adequate total concentration within the soil. Factors affecting the availability and solubility of Zn in soils have been extensively reviewed by several authors (Lindsay, 1972; Kiekens, 1990; Kabata-Pendias and Pendias, 1992). Among the factors affecting Zn availability in soils, many soil parameters, such as total Zn content, pH, organic matter content, adsorption sites, microbial activity and soil moisture play important

roles. Other factors, such as climatic conditions and interactions between Zn and other macro- and micronutrients at the soil and plant level also largely influence Zn availability.

Thorne (1957) reported that the availability of Zn is low when soil pH is above 6.0 and hence Zn deficiency can often be expected in sandy soils that have been limed to pH values of 6.5 and above, or on naturally occurring calcareous soils. Therefore, cereal crops grown on the calcareous alkaline soils of South Australia, where the pH exceeds 6, are prone to Zn deficiency. Zinc is also strongly bound to the sorption complex of the soil as Zn^{2+} , ZnCl^+ or $\text{Zn}(\text{OH})^+$ ions, depending on the soil pH value. Mobility and concentration of Zn in the soil solution are therefore correspondingly low in soils of high pH. Other researchers have noted that soils with a high organic content have low availability of Zn (Shuman, 1980). Zinc ions (Zn^{2+}) are strongly bound in the organic matter of soil. The amount of exchangeable Zn^{2+} for a given total Zn content increases with increasing organic matter content of the soil (Martens *et al.*, 1966). The amount of Zn available to plants can also be influenced by the composition of clay minerals in the soil. For instance, despite high total Zn concentrations, Zn availability in soils with a high montmorillonite content is much lower than in soils containing clay minerals with a low sorption capacity (Thorne, 1957). Clay soils of high magnesium (Mg) content may render Zn unavailable by the adsorption of Zn ions onto the clay minerals in place of Mg. Soils which are high in native phosphate may bind Zn, converting it to an unavailable form (Bergmann, 1992).

It is apparent that the total concentration of Zn in a soil does not necessarily reflect Zn availability to the plant. Therefore, the total Zn concentration of soil is of little value in predicting actual availability to plants, although it provides a source of long-term Zn availability. Zinc present in soil solution is immediately available but alone, cannot meet plant needs throughout the entire plant life span. Therefore, the availability of Zn to growing plants is a function of the capacity of a soil to replace available Zn as it is taken up by plants from soil solution (buffer power) and the rate of replacement of Zn from the soil solid phase to the soil solution (rate of desorption and diffusion) (Dang *et al.*, 1993a).

2.2 Zinc in plants

The predominant form in which Zn is absorbed by plant roots is the Zn cation (Zn^{2+}). However, hydrated Zn and several other complexes and Zn organic chelates may also be absorbed (Kieken, 1990). Absorbed Zn (Zn^{2+}) acts either as a metal component of enzymes or as a functional, structural or regulatory cofactor. Zinc is an essential component or cofactor of many enzymes involved in virtually all aspects of plant metabolism. Zinc has been found to be an integral component of only 4 enzymes, but mediates the action of nearly 300 enzymes in a variety of living organisms (Vallee and Auld, 1990). At least four enzymes in higher plants contain bound Zn: carbonic anhydrase, alcohol dehydrogenase, copper-zinc (Cu-Zn) superoxide dismutase and RNA polymerase (Marschner, 1995). Furthermore, Zn is required as a cofactor for the activity of various enzymes, such as dehydrogenases, aldolases, isomerases, transphosphorylases, as well as RNA and DNA polymerases. Zinc is also required for the synthesis of tryptophan, a precursor for the synthesis of the hormone indoleacetic acid (IAA) (Kieken, 1990).

2.2.1 Zinc and carbohydrate metabolism

Effect on photosynthesis: A decrease in the rate of photosynthesis has been observed under conditions of Zn deficiency (Fujiwara and Tsutsumi, 1962) but direct involvement of Zn in photosynthetic reactions has not been confirmed (Shkol'nik, 1984). However, zinc has been proposed to play a supportive role in maintaining chloroplast structure (Vesk *et al.*, 1966). It is a constituent of phosphoglycerol aldehyde dehydrogenase, and therefore plays a role in the reductive transformations of photosynthetic products (Shkol'nik, 1984).

The first enzyme found to contain Zn was carbonic anhydrase (CA) which catalyses the reversible conversion of carbonic acid to carbon dioxide and water. Other recent suggestions for the role of CA in higher plants include a key enzyme in the metabolism of carbonyl sulphide and as a proton conductor in the plasma membrane of pea leaves (Ignatova *et al.*, 1993). It has been demonstrated that removal of Zn from CA

molecules results in an irreversible loss of enzyme activity (Guliev *et al.*, 1992). When Zn is limiting, CA is suggested to serve as a source of Zn (Rengel, 1995). This Zn pool may be depleted after transfer to Zn-deficient conditions resulting in an eventual loss of CA activity.

Under conditions of Zn deficiency, ribulose biphosphate carboxylase activity in C₃ plants is reduced (Jyung *et al.*, 1972) and in C₄ plants phosphoenol pyruvate carboxylase activity declines (Shrotri *et al.*, 1983). Both of these enzymes receive CO₂ as their substrate from CA. Therefore the reduced activity of the above mentioned enzymes may be considered to be a result of a decline in activity of CA. In contrast, Randall and Bouma (1973) found no close relationship between photosynthesis and CA activity. Their results demonstrate that although CA activity significantly decreased in the Zn-deficient leaves of spinach, only the lowest level of CA activity could reduce photosynthetic rate. Therefore the hypothesis that CA facilitates CO₂ transport into the chloroplasts for the proceeding Calvin cycle was not supported. However, spinach has relatively high CA activity compared with wheat and it is possible that changes in wheat CA activity have a greater influence on photosynthesis.

Effect on carbohydrate partitioning: Besides its function in the carbonic anhydrase reaction, many Zn-dependent enzymes are involved in carbohydrate metabolism in general and in the leaves in particular (Marschner, 1995). The effect of Zn in carbohydrate metabolism is thought to arise due to its role in photosynthesis and phosphorylation (Shkol'nik, 1984).

Zinc is required for the activity of two key enzymes, fructose-1,6-bisphosphatase and aldolase. Fructose-1,6-bisphosphatase is a key enzyme in the partitioning of C₆ sugars in the chloroplasts and the cytoplasm. Under conditions of Zn deficiency the activity of fructose-1,6-bisphosphatase declined rapidly (Shrotri *et al.*, 1983).

Aldolase regulates the transfer of C₃ photosynthates from the chloroplasts into the cytoplasm, and the C₆ sugar transfer to the glycolytic pathway (Marschner, 1995). Under Zn deficiency, aldolase activity is drastically reduced as is apparent from the fact that

measurements of aldolase activity were even once used as an indicator of the Zn status of plants (O'Sullivan, 1970).

Zinc deficiency decreased sucrose levels in sugarbeet by decreasing sucrose synthase activity (Singh and Gangwar, 1973). Zinc deficiency also depressed starch concentration and soluble starch synthetase activity in bean plants (Jyung *et al.*, 1975). Consistent with the observations above, Zn enhanced the starch and sugar concentrations in the leaves of bean and cabbage plants (Sharma *et al.*, 1982; Marschner and Cakmak, 1989).

Effect on respiration: Alcohol dehydrogenase catalyses the reduction of acetaldehyde to ethanol. Under aerobic conditions, ethanol formation takes place mainly in meristematic tissues, e.g. root apices (Marschner, 1995), but in Zn-deficient plants, alcohol dehydrogenase activity decreases. Under anaerobic conditions, such as plants grown in waterlogged or submerged soils, the activity of root alcohol dehydrogenases is twice as high in Zn-sufficient compared with Zn-deficient plants. The lower activity of this key enzyme in anaerobic metabolism may impair root functions considerably (Moore and Patrick, 1988). Zinc deficiency has also been shown to strongly inhibit the principal respiratory pathway (glycolysis-the Krebs cycle) (Paribok, 1972) and electron transport in the mitochondrial respiratory chain (Chvapil, 1973).

2.2.2 Protein synthesis

Zinc regulates protein synthesis through its influence on the metabolism of nucleic acids required for transcription. Concentrations of proteins in Zn-deficient plants are reduced and those of amino acids and amides are increased (Römheld and Marschner, 1991) possibly due to a decrease in transcriptional activity.

Römheld and Marschner (1991) describe an inhibition of protein synthesis in Zn-deficient plants caused by a marked decline in RNA content due to either lower activity of the Zn-containing RNA polymerase (Soloiman and Wu, 1985), reduced structural integrity

of ribosomes (Obata and Umebayashi, 1988), or because of enhanced RNA degradation (Cakmak *et al.*, 1989).

2.2.3 Gene expression

Zinc has structural and regulatory roles in a large number of enzymes and proteins which directly affect replication and transcription processes and gene activation (Vallee and Falchuk, 1993). Zinc is involved in the control of gene expression through its essential role in the so-called 'Zn-finger' proteins (Chesters, 1991). The first of the Zn-finger proteins to be recognised was a transcription factor TFIIIA necessary for the synthesis of the *Xenopus* 5S RNA (Hanas *et al.*, 1983; Klug and Rhodes, 1987). 5S RNA is a constituent of the ribosomes on which molecules of messenger RNA are translated into protein (Rhodes and Klug, 1993). Miller *et al.* (1985) proposed that RNA polymerase contained nine Zn atoms per complex; however, no evidence for the presence of nine atoms was found (Shang *et al.*, 1989). The addition of Zn to the RNA polymerase III containing two intrinsic Zn atoms enhanced neither the binding affinity of the protein for DNA nor transcriptional activity. Under conditions of Zn deficiency, the low efficiency of RNA polymerase III is suggested to cause a general decrease in gene transcription and subsequently translation of proteins (Hanas *et al.*, 1983).

The low protein contents and high amino acid contents in Zn-deficient plants are not only the result of reduced transcription and translation but also of enhanced rates of RNA degradation (Marschner, 1995). Higher rates of RNase activity are a feature of Zn deficiency (Sharma *et al.*, 1982), and an increase in RNase activity is detected before visual symptoms of Zn deficiency occur (Dwivedi and Takkar, 1974).

2.2.4 Plant growth regulators

Zinc also functions in auxin metabolism of plants, although details of the physiological processes involved have yet to be elucidated. One of the symptoms of Zn deficiency is growth retardation, especially of the internode, which is closely associated

with a reduction of auxin concentration in Zn-deficient plants (Shkol'nik 1984). Auxins are formed in shoot tip apices and are translocated down the stem into the root. Auxins are responsible for cell extension of both shoots and roots (Marschner, 1995). Zinc deficiency has been associated with reduced synthesis of the auxin indole acetic acid (IAA) (Römheld and Marschner, 1991).

Biosynthesis of IAA is likely to proceed from tryptophan via the intermediate tryptamine (Marschner, 1995). Higher tryptophan and tryptamine concentrations in young leaves of Zn-deficient plants may indicate an impaired conversion of tryptophan to tryptamine (Römheld and Marschner, 1991) or the conversion of tryptamine to IAA (Takaki and Kusushizaki, 1970). Lower activities of gibberellin-like substances, which are thought to promote the conversion of tryptophan and tryptamine to IAA, have been identified in Zn-deficient maize (Suge *et al.*, 1986).

2.2.5 Activated oxygen species metabolism

Zinc plays an important role in preventing the destruction of cellular components by activated oxygen species. A predominant activated oxygen species is the superoxide radical, ($O_2^{\cdot -}$), which is formed in living cells during several biochemical reactions. Superoxide dismutases (SOD) are metalloproteins catalysing the conversion of superoxide free radicals to molecular oxygen and H_2O_2 (Giannopolitis and Ries, 1977). Living cells use this mechanism to prevent an excessive accumulation of superoxide free radicals which would otherwise cause cell death (McCord and Fridovich, 1969).

Superoxide dismutase activity was first described in a cupro-zinc protein (erythrocuprein) from bovine erythrocytes (McCord and Fridovich, 1969). Similar cupro-zinc proteins with SOD activity have subsequently been isolated from various eukaryotic sources (Fridovich, 1974). The Cu-Zn SOD enzyme of plants is present in the chloroplasts of the green tissue, in the cytosol of nongreen tissue, and a small amount in the space between the inner and the outer mitochondrial membranes (Halliwell, 1982). The role of Zn is crucial to the regulation of superoxide free radical levels in the cells. Consequently,

Zn-deficient plants have been found to contain low levels of superoxide dismutase activity and, therefore, high levels of superoxide free radicals (Vaughan *et al.*, 1982; Cakmak and Marschner, 1988b,c). Elevated levels of toxic oxygen free radicals in Zn-deficient plants may destroy the double bonds of polyunsaturated fatty acids and phospholipids in the membranes, and increase leakage of solutes such as K^+ , sugars, and amino acids (Cakmak and Marschner, 1988a).

2.2.6 Membrane integrity

In higher plants, Zn is required for maintaining the integrity of membranes (Cakmak and Marschner, 1988a; Welch and Norvell, 1993; Welch *et al.*, 1982). This requirement in plant cell membranes is attributed to the capacity of Zn to interfere with the generation of oxygen radicals by an NADPH oxidase as well as being a metal component of the oxygen-detoxifying superoxide dismutase (SOD) (Cakmak and Marschner, 1988b,c). Elevated levels of toxic oxygen radicals could, in turn, be responsible for the changes in amounts and composition of phospholipids and increases in membrane permeability in Zn-deficient root cells (Cakmak and Marschner, 1988a; Welch and Norvell, 1993; Pinton *et al.*, 1994). Therefore, a major role of Zn in membrane stabilisation is to control the level of oxidising oxygen species. Due to preferential binding of Zn to thiol (-SH) groups, Zn plays a key role in the stabilisation and maintenance of structural orientation of particular membrane proteins (Römheld and Marschner, 1991). Zinc binds with the thiol (-SH) groups of the membrane proteins to form stable mercaptides which are much less susceptible to oxidation to disulphides by activated oxygen species than unprotected -SH groups (Warren *et al.*, 1966).

Zinc is responsible for the stabilisation of cell membranes through its interaction with phospholipids (Sunamoto *et al.*, 1980; Bettger and O'Dell, 1981; Von Glos and Boursnell, 1981). Zinc deficiency may not only reduce the integrity of the plasma membrane but probably also other membranes such as the tonoplast.

2.3 Zinc deficiency

Zinc deficiency in agricultural crops is one of the most common micronutrient deficiencies in southern Australia. Zinc deficiency can be expected on the following soil types (Bergmann, 1992):

- Acid leached sand and sandy loam soils, particularly on podzols with a low Zn content and especially after heavy phosphorus fertilisation and /or liming.
- Neutral and carbonate soils with low available Zn contents, poor Zn mobility and high levels of available phosphorus and organic matter,
- Soils containing a great deal of organic matter that decomposes slowly,
- “Ameliorated” soils where a large amount of subsoil with a poor Zn status has been mixed with the topsoils, and
- Planted soils where some of the topsoil has been removed.

It can thus be concluded that Zn deficiency arises from three main causes: low content of total Zn in the soil; unavailability of Zn present in the soil to the crop and management practices that depress the availability or uptake of Zn.

Screening for Zn efficiency in Australian cereal cultivars began after marked increases in the incidence of Zn deficiency in crops. Farmers changing from the traditional superphosphate as their phosphorus fertiliser to the new high-analysis products such as diammonium phosphate precipitated this. The latter contains much less Zn as a natural contaminant.

2.3.1 Symptoms of zinc deficiency

Signs of Zn deficiency in wheat are usually localised on the middle leaves and extend to the new growth. The degree and extent of the symptoms depend on the severity of the deficiency (Brennan, 1983).

One symptom suggestive of mild Zn deficiency is a longitudinal pale green stripe on each side of the mid-vein of fully emerged leaves. The leaf tissue in this stripe soon dies and the necrotic (dead) area turns a pale brown. In some plants this stripe may occur on only one side of the mid vein. Necrotic patches usually appear along these stripes. The

leaf changes from green to a muddy greyish-green in the central areas of the middle leaves. As the deficiency becomes more severe, there is a general paling (chlorosis) of the leaves. Stunted plants often have "succulent-looking" leaves or a "diesel-soaked" appearance, showing necrotic areas about halfway along the leaves, causing them to bend in the middle section. The necrotic areas increase and are surrounded by yellow-mottled areas. At this stage the middle leaves collapse in the centre, resulting in drooping leaf tips. These symptoms are generally more advanced in the older leaves (Grundon, 1987).

While grain yields may be greatly reduced in severe cases of Zn deficiency, near maximum yields can be produced by plants displaying mild vegetative symptoms. Seedlings and plants affected by mild Zn deficiency early in the season, when soil conditions are cool and wet, tend to recover naturally in drier, sunnier conditions of spring.

Plants affected by mild Zn deficiency can be diagnosed by determining the concentration of Zn within plant tissue. Several authors have suggested values for critical Zn concentrations in plant tissue below which responses to applied Zn would be expected. Diagnostic plant tissue tests show that critical Zn concentrations vary with the particular plant part analysed (Table 1.1) (Dang *et al.*, 1993b).

Table 1.1. Critical Zn concentrations, corresponding to a relative yield of 90%, estimated in different plant sections of wheat, with Zn application rates ranging from 0 to 30 kg ha⁻¹ equivalent. (Reproduced from Dang *et al.*, 1993b).

Plant section	Critical Zn concentration
	mg kg ⁻¹
Youngest mature blade	16.0
Old leaves	12.5
Ear	31.0
Stem	9.4
Whole top	16.4

2.3.2 Correction of zinc deficiency

Several reviews discuss the effects of agronomic practices on the nutritional quality of major food crops with respect to Zn (Lindsay, 1972; Welch, 1995). The most efficient agronomic practice currently available to farmers to increase Zn concentration of crops and hence final yields is the application of Zn fertilisers (Welch and House, 1984). In addition, Zn-efficient cultivars (those that are able to extract and utilise zinc from a Zn-deficient soil to a greater extent than other cultivars) can also help (Graham and Rengel, 1993).

As Zn is known to be essential for plant growth, there has been surprisingly little work done on the interaction of Zn and disease at or near conditions of Zn deficiency.

2.4 Phosphorus

Phosphorus is essential to plants and P deficiency affects all aspects of growth, because of the role of adenosine triphosphate (ATP) in many aspects of metabolism. Poor root growth and the failure to absorb other minerals as ions are the most immediate influential outcomes of phosphorus deficiency.

2.4.1 Elemental phosphorus

Phosphorus (P) is a nonmetal, being a non-conducting element that tends to form negative ions far more readily than positive ions.

Phosphorus is the second member of the nitrogen family but contrasts sharply with nitrogen in its natural chemistry. Phosphorus atoms do not have triple bonds between them but instead have single bonds which can form chains and rings. There are three allotropes of P (red, yellow (white) and black) which reflect the different molecular formulas and geometrical organisations.

2.4.2 Phosphorus in soils

Total phosphorus content of Australian soils may vary from 0.02 to 0.5%, with an average of approximately 0.05% (Barber, 1995). The widespread occurrence of P

deficiency in Australian soils is the result of several unusual features of the geological and pedological histories of the continent, heading to the development of many strongly leached, highly weathered soils of low fertility, together with younger soils which have been developed from parent materials affected by earlier P-depleting weathering cycles (Taylor, 1983). Phosphorus is present in four general forms: (1) phosphorus as ions and complexes in the soil solution (Kovar and Barber, 1982); (2) phosphorus adsorbed on the surfaces of inorganic soil constituents (Barber, 1995); (3) phosphorus minerals, both crystalline and amorphous (Lehr, 1980); and (4) phosphorus as a component of soil organic matter (Larson *et al.*, 1972). A large fraction of the P present is in a mineral form not readily available for absorption by the plant.

2.4.3 Plant availability of phosphorus in soils

Plants absorb phosphorus preferably in the form of phosphate ion HPO_4^{2-} and less readily as H_2PO_4^- (Marschner, 1995). Soil pH controls the relative abundance of these two forms, H_2PO_4^- being favoured below pH 7 and HPO_4^{2-} above pH 7 (Salisbury and Ross, 1985). Soil organic P slowly becomes mineralised into inorganic P, and it is in this form that plants generally take it up through their root systems from the soil solution (Sauchelli, 1965). Much phosphate is converted into organic forms upon entry into the root or after transport as inorganic P through the xylem. Acid and alkaline phosphatases are responsible for the conversion of organic P to inorganic P in the final stage of the mineralisation process of organic P fractions (Rao *et al.*, 1996). Acid and alkaline phosphatases are suggested to dominate in acid and alkaline soils, respectively (Dick and Tabatabai, 1984).

2.5 Phosphorus in plants

Phosphorus is present in plant tissues in both organic and inorganic forms and is indispensable for all forms of life because of its structural role in the backbone (sugar-phosphate) of ribonucleic acids and function in energy transfers via adenosine triphosphate

(ATP). Phosphorus is needed in cell division, in the formation of fat, in the transformation of starch to sugar, in seed germination, in fruiting and flowering, and in fact in every phase of all vital processes (Sauchelli, 1965). Phosphorus is a mobile element within the tissues, and in a growing plant, it is transferred or redistributed from one organ to another, particularly when P may be in short supply in the soil. In this situation P is translocated from the older leaves to newer, growing tissues and especially to developing seeds. The P requirement for optimal growth is in the range of 0.3-0.5% of the plant dry matter during the vegetative stage of growth (Marschner, 1995).

2.5.1 Phosphorus as a structural element

The function of P as a constituent of macromolecular structures is most prominent in nucleic acids, which, as units of the DNA molecule, are the carriers of genetic information and, as units of RNA, are the structures resulting from transcription and required for translation of the genetic information (Marschner, 1995). In DNA each base is chemically linked to deoxyribose, forming a compound called a nucleoside. A phosphate group is also attached to the sugar of each nucleoside, yielding a nucleotide.

The precursors in the synthesis of RNA are four ribonucleoside 5' -triphosphates, and the chemical reaction that occurs to form RNA is the same as that in the synthesis of DNA, though the enzyme is different.

2.5.2 Carbohydrate metabolism

Effect on photosynthesis: The light energy trapped in the initial reactions of photosynthesis is eventually converted into the chemical energy contained in stable, reduced carbon compounds principally carbohydrates. ATP, which acts as an intermediate energy transfer compound in such cell functions as photosynthesis, respiration, stomatal opening, and the transfer of organic and inorganic solutes across membranes, requires inorganic phosphate in its formation from ADP (Terry and Ulrich, 1973).

Photosynthesis is inhibited in plants grown with an insufficient supply of inorganic P (Brooks *et al.*, 1988). Early research suggested that photosynthetic CO₂ fixation in P-deficient plants may be limited by a diminished photosynthetic electron transport capacity, or an insufficient inorganic P supply available for the phosphorylation of ADP to ATP (Terry and Ulrich, 1973). Evidence that P deficiency affects ATP and NADPH was based on the fact that low P decreases the photosynthetic activity and the rate of ATP and NADPH formation of *Beta vulgaris* chloroplasts (Tombesi *et al.*, 1969). In contrast, low P has also been shown to decrease photosynthesis through an effect on ribulose 1,5-bisphosphate regeneration, this effect apparently not being due to ATP and NADPH limitation (Rao *et al.*, 1989).

Effect on carbohydrate partitioning: Phosphorus deficiency diminishes photosynthetic CO₂ fixation rates (Terry and Ulrich, 1973) and the expansion of the leaf surface (Sommer, 1936). It may also lead to a decreased cytosolic orthophosphate level (Treeby *et al.*, 1987). Orthophosphate is thought to regulate the activities of several enzymes involved in starch and sucrose metabolism *in vivo* and the export of carbon out of the chloroplast via the orthophosphate translocator (Daie, 1985; Freeden *et al.*, 1989). An increase in starch and a subsequent decrease in sucrose concentration in P-deficient leaves was correlated primarily with changes in the total activities of enzymes of starch and sucrose metabolism and may not necessarily be mediated via the orthophosphate translocator (Freeden *et al.*, 1989).

2.5.3 Phosphorus deficiency

Phosphorus, as stated previously, is an important nutrient in crop production on Australian soils, since many soils in their native state do not contain sufficient available phosphorus to maximise crop yield (Barber, 1995). Phosphorus deficiency can be expected on the following soil types (Grundon, 1987):

- Soils low in organic matter;

- Soils where cropping has exhausted the store of P;
- Highly weathered, iron-rich acid soils where phosphate is fixed in unavailable forms;
- Soils where the topsoil has been lost through erosion; and
- Calcareous soils where much of the P may be tied up in insoluble calcium (Ca) phosphates which are poorly available to plants.

2.5.4 Symptoms of phosphorus deficiency

The symptoms of P deficiency are usually first seen on older leaves, as P is transferred from older to younger leaves. The older leaves remain dark green but develop a dark yellow chlorosis at the tip which develops into a dark brown necrosis that advances down the leaf, usually along the leaf margins (Grundon, 1987). Eventually, affected leaves die and turn brown to form a thatch of dead leaves around the base of the stem. The younger leaves remain dark green but may be shorter and more erect than usual.

In plants suffering from P deficiency, reduction in leaf expansion and leaf surface area (Freedon *et al.*, 1989), and also number of leaves (Lynch *et al.*, 1991), are the most striking effects. Root elongation is much less inhibited under P deficiency, leading to a typical decrease in shoot-root dry weight ratio (Marschner, 1995). The change in shoot-root ratio is correlated with an increase in partitioning of carbohydrates towards the roots. Phosphorus deficiency may enhance the elongation rate of individual root cells and of the roots (Anuradha and Narayanan, 1991).

2.5.5 Correction of phosphorus deficiency

The growth of crops depends on the amount of water-soluble phosphates and the rate of exchange between soluble and insoluble forms of phosphorus in the soil (Grundon, 1987). Phosphorus deficiency can be corrected by applying phosphatic fertilisers to the soil at or before sowing.

2.6 Genetic variability

Genetic variability in relation to yield is defined as the heritable characteristics of a plant species or cultivar that makes it differ in yield potential in unfavourable environments as compared to other species or cultivars within species (Fageria *et al.*, 1991). Useful genetic variation apparently exists in all major field crops and cultivars within crops for tolerance to mineral deficient conditions (Duvick *et al.*, 1981; Lafever, 1981; Epstein and Rains, 1987), diseases (Day, 1973; Buddenhagen, 1983), insect attacks (Beck, 1965), and drought tolerance (Bruckner and Frohberg, 1987). Due to genetic variability, some plant species or cultivars adapt and produce a greater yield under particular environmental conditions than other species or cultivars. Advantage may be taken of these differences to improve plants for growth under defined or constrained fertiliser conditions and for the adaptation of plants to mineral deficient conditions (Clark, 1983).

Exploiting the genetic variability of agricultural crops is justified if four criteria are satisfied (Mahon, 1983):

1. The variability must be of sufficient magnitude to justify the cost of assessing the character.
2. If selection for improved expression of a characteristic is to be useful over a range of spatial and temporal environments, some stability of genotype performance is necessary.
3. The variability must be related to agronomic benefit, either in increasing yield or quality or reducing cost of production.
4. The variability must be measurable in large-scale trials.

Plant species or cultivars which produce a greater yield than another cultivar grown in the same unfavourable environmental conditions do not necessarily produce a greater yield under favourable conditions and vice versa. Therefore, in selecting a species or cultivar under unfavourable conditions, the objective should be to stabilise productivity. Yield stability is a measure of variation between the potential and the actual yield of a cultivar across changing environments (Blum, 1980), which can result from genetic

heterogeneity, yield component compensation, stress tolerance, capacity to recover rapidly from stress, or a combination of these factors (Heinrich *et al.*, 1983).

Therefore cultivars with traits for increased uptake, translocation, accumulation and use of nutrients can be termed nutrient efficient or tolerant of nutrient deficiency.

2.6.1 Cultivar differences in nutrient efficiency

Plant species differ extensively in the uptake, translocation, accumulation, and use of mineral elements (Clark, 1983). Diversity among cultivars within a plant species for mineral element uptake, translocation, distribution, and use have been recognised for many years (Gerloff, 1963; Brown *et al.*, 1972; Jung, 1978; Devine, 1982; Graham, 1984).

The differences in nutrient uptake have most commonly been evaluated by growing plants in soils of known nutrient deficiency or in nutrient solutions differing in concentration for a given nutrient element, and determining a yield response curve (dry matter production or grain yield) (Graham, 1984; Graham *et al.*, 1992).

One important determinant of seedling growth is the amount of reserves contained in the seed (Bremner *et al.*, 1963). Care must be exercised in comparing cultivars for nutrient efficiency. One needs to ensure the seed of all cultivars has approximately the same nutritional content, otherwise differences in seed content of the limiting nutrient can contribute to seedling vigour and confound the interpretation of genetically based differences (Uren *et al.*, 1988). The importance of early seedling growth for the successful establishment of crop species has been recognised for many years (see De Marco, 1990; Rengel and Graham, 1995). Although seed energy reserves are vital to the growth of a seedling, particularly during its pre-photosynthetic period, the importance of protein and mineral reserves to early seedling growth has been demonstrated (De Marco, 1990). Larger seeds grown in a highly P-deficient sand culture system were shown to produce larger leaves, heavier plants and longer roots than plants grown from smaller seeds (De Marco, 1990).

Genetic variability within crop species has been discussed in Section 2.3, and nutrient efficiency has been defined. Plant species differ widely in Zn (Graham and Rengel, 1993) and P efficiency (Föhse *et al.*, 1988), and efficient cultivars can be identified in breeding programs. Nutrient-efficient cultivars can be described as those that grow and yield well on soils low in available nutrients compared with other cultivars that do not grow as well. Differences in cultivar tolerance to several soil-borne diseases have been associated with their differences in efficiency of nutrient uptake, a relationship presumably due to enhanced nutrient concentrations (Graham and Webb, 1991).

2.7 *Rhizoctonia solani* Kühn

Rhizoctonia solani (*R. solani*) Kühn was first identified more than 100 years ago by Kühn in 1858. *Rhizoctonia* patch or 'bare patch' was first recorded in Australia by Samuel (1928) who reported a *Rhizoctonia* species as causing seedling disease in wheat and oat fields in South Australia. Samuel and Garrett (1932) later gave a description of the disease. An occurrence of *R. solani* in New South Wales was reported in 1933 (Hynes, 1933) but the disease was not identified positively in Western Australia until 1971 (MacNish, 1983).

2.7.1 Taxonomy of *Rhizoctonia solani*

Rhizoctonia solani Kühn

Class: Basidiomycetes

Order: Aphyllophorales

Family: Tulasnellaceae

Stat. Basid: *Thanatephorus cucumeris* (Frank) Donk (Domsch and Gams, 1972)

T. cucumeris is the teleomorphic state (sexual stage) of the multinucleate anamorph (asexual stage) *R. solani*.

In general, researchers have recognised *R. solani* as being characterised by (Parmeter and Whitney, 1970; Carling and Sumner, 1992):

1. Branching near the distal septum of young vegetative hyphal cells,
2. Constriction of branch hyphae at the point of origin,
3. Formation of a septum in the branch near the point of origin,
4. Absence of clamp connections, conidia and rhizomorphs,
5. Presence of the dolipore septal apparatus, and
6. Sclerotial tissue not differentiated into a rind and medulla.

It is the multinucleate anamorph (*R. solani*) which is responsible for plant disease. Isolates of *R. solani* are extremely variable in cultural morphology and host range (Schneider *et al.*, 1997). *Rhizoctonia solani* is considered a species complex rather than a single species (Anderson, 1982). The species complex can be subdivided by means of hyphal anastomosis reactions between isolates into more homogeneous groups called anastomosis groups (Ags) (Schneider *et al.*, 1997). To date, twelve anastomosis groups of *R. solani* have been described (AG-1 to AG-11 plus AG-B1) (Sneh *et al.*, 1991; Carling *et al.*, 1994). The anastomosis group 8 (AG-8) is the soil-borne plant pathogen that induces bare patch in cereals (Neate and Warcup, 1985). This group can be further divided into pectic zymogram groupings, of which 5 groups have been identified, ZG1-1 to ZG1-5. A comparison of anastomosis to zymogram groups has been conducted and isolates in AG-8 fell into ZG1-1, ZG1-2, ZG1-3, ZG1-4 and ZG1-5 (Neate *et al.*, 1988; MacNish and Sweetingham, 1993).

Rhizoctonia solani, in pathogenic form, infects a wide variety of host plants causing severe damage in many agricultural and horticultural crops worldwide (Ogoshi, 1987; Sneh *et al.*, 1991). *Rhizoctonia solani* is an endotrophic fungus, meaning that it penetrates the cells of the host, causing root rots, stem cankers, seed decay, aerial blights, damping off, pod rots, and fruit decay (Table 1). Losses of 25% (MacNish, 1986) or more (MacNish and Neate, 1996) have been recorded in cereals in Australia, and the disease has

the potential to be moderate to severe in all cereal cropping areas (Murray and Brown, 1987).

2.7.2 Distribution

The disease caused by *R. solani* is known by a variety of names world-wide. It causes brown patch of turf grasses in New Zealand (Christensen, 1979) and in North Carolina (Martin and Lucas, 1984); the stunting of clovers in Ontario (Benedict, 1953); barley stunt disorder in Scotland (Murray, 1981), and the United Kingdom (Murray and Nicolson, 1979); bare patch of cereals in Australia (Samuel and Garrett, 1932; MacNish, 1983); crater disease (stunting) of wheat in South Africa (Deacon and Scott, 1985); root rot of wheat and barley in the Pacific Northwest of the United States (Ogoshi *et al.*, 1990); and foot rot of cereals in Japan (Ogoshi and Ui, 1985). The strain AG-8 has been identified in all countries mentioned above, except Japan.

2.7.3 Colonisation and growth of *R. solani* in soil

Rhizoctonia solani Kühn forms sclerotia in soil, and survives for a long period in the absence of a host, either as sclerotia or thick-walled brown hyphae in plant debris (Naiki, 1985). The importance of sclerotia on debris particles and moniloid cells (short thick-walled hyphae) within plant debris, as sources of inoculum of *R. solani* in soil have been demonstrated (Boosalis and Scharen, 1959). However, no sclerotia in soil have been found in Australian soils (Warcup, 1957; deBeer, 1965; Warcup and Talbot, 1962). In contrast to other strains of *R. solani*, the anastomosis group AG-8 does not produce sclerotia in nature (Neate, 1987). It survives in soil as hyphae or as resistant cells inside organic debris (dormancy). The percentage of particles containing hyphae and moniloid cells of *R. solani* decreases with increasing soil depth and increasing sieve size. *Rhizoctonia solani* is found most frequently in the top 10 cm of the soil profile (Papavizas, 1970; Neate, 1987).

Little is known about the nature of the materials stimulating the growth of *R. solani*; however, carbohydrates and amino acids, commonly found in large quantities in the exudates from the seeds and roots of many plants, are most likely to stimulate the development of the fungus (Dodman and Flentje, 1970).

2.7.4 The infection process

Penetration of *R. solani* hyphae into plants may occur directly through the intact cuticle and epidermis, or the fungus may penetrate through natural openings and through wounds (Dodman and Flentje, 1970). Penetration could also occur by invasion of cortical tissues exposed by secondary root emergence (Wyllie, 1959).

Chemical stimuli control the development of infection structures on the host surface by the teleomorphic state of *R. solani* (*T. cucumeris*) (Flentje *et al.*, 1963). Nematodes (*Heterodera schachtii*) not only provide avenues for *R. solani* entry into tissue of sugarbeet and an improved substrate for initial colonisation deep into the cortex, but also reduce the efficiency of cellular defence against fungal invasion (Polychronopoulos *et al.*, 1969).

2.7.5 Disease symptoms

Diseased plants are noticeable at a very early stage, frequently before the crop has begun to tiller. They appear spindly and stunted, with stiff, rolled leaves pointing upwards. The affected plants do not tiller and usually remain developmentally dormant during the winter months, and as the weather becomes warmer, all the plants in many of the patches die. Some plants do recover as the soil gets warmer but are delayed in reaching maturity. The most characteristic symptoms of *R. solani* infection are observable on the roots of cereals. If diseased plants are examined in the earliest stages, the tips or intermediate portions of the root are observed to have lost turgidity. The cortex begins to rot away and the central cylinder breaks, so that the affected part appears as a brown stub. This process

causes brown sunken lesions on the root cortex and, when disease is severe, the stele is completely rotted, leaving characteristic sharp-pointed roots (spear tips) (Neate, 1989).

As the fungus kills the root tips of primary roots, lateral roots are stimulated to form and these are in turn attacked. The result is a rather characteristic, much-branched root system in diseased plants, mainly in the seminal roots. The destruction of primary roots from the first node is, in fact, largely the reason for the severe effect of the fungus on the plants, for they are unable to establish any secondary root system. By the time the root has rotted back to a stump, it is almost impossible to find any active-looking hyphae at all; it is therefore difficult to isolate the fungus during later stages of the disease (Samuel and Garrett, 1932).

2.7.6 Conditions favouring the disease

Low soil temperatures in the range of 12-18 °C (Samuel and Garrett, 1932) favour infection by *R. solani*. At high temperatures, infection is favoured by low levels of soil moisture (Hynes, 1937). *Rhizoctonia solani* is more prevalent on the light, alkaline, sandy, mallee-type soils of pH 8.0-9.0 in South Australia and the lime-deficient soils of pH 6.0-6.3 in New South Wales (Samuel and Garrett, 1932; Hynes, 1937).

Pre-sowing tillage is an effective method of control (Rovira, 1986), but bare patches still appear in some paddocks despite repeated cultivation. Soil disturbance reduces the virulence of *R. solani* (Rovira, 1986; MacNish, 1984; Smith and Wehner, 1988), as tillage breaks the fungal filaments into smaller, less infective pieces. Many farmers in southern Australia have adopted minimum tillage because it reduces the impact of soil erosion, but the highly infective propagules of the fungus remain intact. *Rhizoctonia* infection reduces the total length of roots, which may lead to nutritional deficiencies, hence causing stunting and chlorosis of plants. If fertility is high, plants may be able to cope better with the damage. If conditions are optimal for crop growth, the roots may be able to “grow away” from the fungus.

Rhizoctonia bare patch can be aggravated on alkaline soils by chlorsulfuron (Glean), the sulfonylurea herbicide that is persistent under alkaline conditions. The effect can be observed in crops sown a year after the herbicide treatment (Rovira and McDonald, 1986).

2.7.7 Control measures

Tillage is defined as the mechanical manipulation of soil for crop production (cultivation), and conservation tillage is any tillage sequence which reduces loss of soil water and soil erosion relative to conventional tillage. Until the introduction in South Australia of conservation tillage in its various forms such as direct drilling, no-till, reduced tillage or minimum tillage, *R. solani* occurred only in highly calcareous sandy soils. With the development of suitable herbicides, conservation tillage has become a practical reality for rain-fed cereal farming in Australia, as the soil requires less working to be rid of weeds. The main features of conservation tillage are increased soil surface roughness and maintenance of crop residues on the soil surface. This system of tillage controls wind and water erosion (Roget *et al.*, 1987), reduces energy use, and conserves soil and water. Reduced tillage practices also allow the possibility of grazing annual pasture of grasses and pasture legumes regenerated by autumn rains. With the introduction of conservation tillage there have been several reports of increased crop damage by *Rhizoctonia* and the appearance of the disease outside the traditional areas of calcareous sandy soils (MacNish, 1985; Weller *et al.*, 1986; Rovira, 1986).

2.8 Interaction between plant nutrition and incidence of plant disease

Nutrients influence host-pathogen interactions and have a wide range of effects on plant-disease interactions (Table 2.1) (Graham, 1983; Graham and Webb, 1991). The addition of a particular nutrient to deficient soil may affect the status of the plant, the pathogen, or both, thus affecting the host-pathogen relationship. The effect of many nutrients on disease has been observed because a nutrient deficiency condition was present

which required nutrient amendment for optimum plant growth. Thus, the effect of nutrients on disease was incidental to fertiliser programs designed to optimise plant growth, with the observed field responses studied in the laboratory under more controlled conditions later (Huber and Watson, 1970; Smiley and Cook, 1973).

Mineral elements are directly involved in all mechanisms of a plant's defence against disease as integral components of substrates, enzymes, and electron carriers; or as activators, inhibitors, and regulators of metabolism (Huber and Graham, 1998). The effects of nutrients on disease may be attributed to (Fageria *et al.*, 1991):

1. Effects on plant growth that can influence the microclimate in a crop and thereby affect infection and sporulation of the pathogen.
2. Effects on cell walls and tissues, as well as on the biochemical composition of the host.
3. The rate of growth of the host, which may enable seedlings to escape infection in their most susceptible stage, and
4. Effects on the pathogen through alterations in the soil environment (Colhoun, 1973).

A direct toxic effect of high soil Zn on the pathogen living as a saprophyte in a soil nutrient medium has been discarded by Thongbai *et al.* (1993c), and it is hypothesised that the suppression of *R. solani* disease must be due to the physiological effect of Zn on the cereal plant. Another hypothesis is that Zn-adequate plants may have larger root systems that increase the plants' nutrient uptake potential. Differences in cultivar tolerance to several soil-borne diseases have been associated with their differences in efficiency of nutrient uptake, a relationship presumably due to enhanced nutrient concentrations in infected plants (Graham and Webb, 1991).

Disease suppression through manipulation of nutrient availability may be achieved by direct application of a nutrient, by cultural practices which modify abiotic environments influencing nutrient availability, and by modifying the plant genotype relative to its nutrient uptake capacity or interaction with the abiotic or biotic environment (Huber and Graham, 1998).

Table 2.1 List of diseases which are caused by *R. solani* and influenced by elements (Huber, 1980)

Disease	Element	Host plant	Effect after adding nutrient
Damping off	Calcium	Soybean	Decrease
		Bean	Decrease
Damping off	Phosphorus	Pea/cucumber	Decrease
Damping off	Magnesium	Cotton	Decrease
Root rot	Magnesium	Bean	Decrease
		Soybean	Decrease
Root rot	Sulfur	Soybean	Decrease
Root rot	Sodium	Bean	Increase
Root rot	Iron	Soybean	Decrease
Root rot	Phosphorus	Soybean	Decrease
Root rot	Potassium	Jute	Decrease
Canker	Potassium	Potato	Decrease
Pod rot	Calcium	Bean	Decrease
		Cereal	Decrease
		Soybean	Decrease
Pod rot	Magnesium	Peanut	Increase
Stem canker	Manganese	Potato	Decrease
Stem canker	Copper	Potato	Decrease

Since Hubers report in 1980 (see Table 2.1), other nutrients have also been found to reduce the incidence or severity of *R. solani*. The incidence of black scurf (*Rhizoctonia solani*) of potato was depressed from an average of 97 % to an average of 12 % by dipping the tubers in 0.05 % ZnSO₄ plus 1 % acetic acid for 15 minutes before planting (Somani, 1986). Both zinc and nitrogen have been demonstrated to play a role in reducing the severity of infection by *R. solani* in wheat (Thongbai *et al.*, 1993a, Wall *et al.*, 1994).

2.8.1 Interactions between plant nutrition and incidence of disease caused by *R. solani*

Studies have shown that Zn deficiency predisposes plants to attack by fungal pathogens (Somani, 1986; Thongbai *et al.*, 1993a,b). In an area responsive to Zn fertilisation, root-rot diseases of wheat were more prevalent on plants grown without added Zn than on those grown with 35 kg ha⁻¹ Zn sulphate (ZnSO₄) (Millikan, 1938). In a field experiment conducted in 1989 and 1990, the recommended rate of 2.5 kg Zn ha⁻¹ reduced the area affected by *Rhizoctonia* (bare patch) from 42% to 21% of the total crop area (Thongbai *et al.*, 1993a). The results showed that Zn-deficient plants appeared to be more sensitive to *Rhizoctonia* root rot. Therefore, cultivars of wheat that are able to extract and utilise zinc from the soil to a greater extent than other cultivars should be studied for their role in the suppression of *R. solani*.

The elements Zn and P are both to be used in the following experiments because internal requirement for these elements in different cultivars differs dramatically. This experimental approach is directed at comparing nutrient-deficient wheat cultivars with nutrient-sufficient wheat cultivars to determine their relative susceptibility to *R. solani*.

2.9 Research aims

- To determine a growth system that will allow both a nutrient regime and the colonisation of *R. solani* to be successful.
- To compare the susceptibility of wheat to *R. solani* by comparing Zn and P deficiencies.
- To determine if root parameters can be used to compare the severity of infection over nutrient regimes.
- To confirm if Zn-deficiency predisposes wheat to infection by *R. solani*.
- To compare the susceptibility to *R. solani* between cultivars under Zn and P deficiency.

Chapter 3

Preliminary experiments on the effects of Zn nutrition on *Rhizoctonia solani* Kühn (AG8) infection of wheat

3.1 Introduction

The fungus *Rhizoctonia solani* (AG 8) is responsible for causing bare patch in wheat (de Boer and Kollomorgen, 1987; MacNish, 1983), a disease which is common on light calcareous and/or siliceous sandy soil (Dubé *et al.*, 1987) in much of the Australian cereal-growing areas. Light calcareous and/or siliceous sandy soils can be chronically zinc-deficient (Thorne, 1957) and are easily eroded under heavy tillage. Minimum tillage is recommended in these situations and is being increasingly adopted by farmers. However, these areas are reported to be severely affected by *Rhizoctonia* (Dubé *et al.*, 1987), and minimum tillage has been associated with the increase in disease severity (Rovira, 1986).

The application of micronutrient cations to deficient soils has been demonstrated to increase tolerance and resistance to disease in many plants (Millikan, 1938; Graham and Webb, 1991; Thongbai *et al.*, 1993a). Unlike other micronutrient deficiencies, Zn deficiency is a common feature of both cold and warm climates as well as acid and alkaline soils of either heavy or light texture (Graham *et al.*, 1992). Therefore, in southern Australian soils, which are typically zinc deficient and can have large infestations of *R. solani*, the application of Zn fertiliser may be of benefit in reducing the yield losses associated with *R. solani* infection.

In the last sixty years much research has been conducted on the effect of micronutrient application on the severity of root-rot diseases. Small applications of Zn sulphate to various cereal species has been shown to give a marked increase in growth and yield of wheat and oats, and a decrease in the severity of root-rot diseases (Millikan, 1938).

However, in these studies, the Zn status of the plants and of the soil was not measured nor was the effect of Zn on disease severity quantified. Somani (1986) reported an effect of Zn in decreasing potato black-scurf caused by *R. solani* of a different anastomosis group to that infecting wheat. The application of Zn to Zn-deficient soil has also been associated with decreased levels of fungal infections of wheat. Zinc applied to Zn-deficient soil decreased the level of *Fusarium* infection in wheat (Grewal *et al.* 1996). Similarly, the application of Zn to Zn-deficient soil decreased the severity of *Rhizoctonia solani* root rot in the hexaploid wheat cultivar Songlen (Thongbai *et al.*, 1993a).

Small differences in tolerance to *R. solani* (AG-8), both within and between the cereal genera have been observed, although the differences were too small to either be useful to breeders or affect rotations (Neate, 1989). Therefore, the question arises as to whether tolerance of wheat cultivars to *R. solani* is affected by the Zn efficiency of the given cultivar in relation to the amount of Zn applied. In order to address this question, it is necessary to first determine environmental conditions which optimise the Zn-*R. solani* relationship in wheat cultivars.

To ascertain suitable conditions for investigating the relationship between the host (wheat) and the pathogen (*R. solani*), factors such as uniformity of the *R. solani* inoculum (McDonald and Rovira, 1985), the required number of replications (Rovira *et al.*, 1985; Wilhelm *et al.*, 1988; Sparrow and Graham, 1988) and the rates of Zn application (Sparrow and Graham, 1988; Thongbai *et al.*, 1993b) need to be evaluated. The system needed to study nutrient-pathogen interactions is, by nature highly variable and all factors concerning this variability must be dealt with.

The procedure developed by McDonald and Rovira (1985) of providing uniform inoculum of *R. solani* within pots, was tested by Thongbai *et al.* (1993b) and appeared successful. In the study by Thongbai *et al.* (1993b), colonising soil with *R. solani* was achieved by using either millet seed or wheat bran as a substrate. *Rhizoctonia solani* inoculum on wheat bran has been shown to cause a larger range of disease severity compared to the inoculum on millet seed (Thongbai *et al.*, 1993b). However, millet seed is the preferred substrate when investigating mechanisms of infection caused by *R. solani* and

not a range of infection levels as a more even level of infection is observed (S. M. Neate, pers. commun.).

The level of available Zn contained in soil varies with soil type and the weather conditions at the time of soil collection. In order to obtain Zn-deficient conditions for plant growth in soil, several application rates of Zn need to be tested to establish an experimental system for a nutrient-disease interaction. The level of applied Zn also varies with the disease in question. The Zn nutritional status of a host has been shown to influence disease resistance or tolerance only in the range of deficiency and is not evident when the Zn nutritional status is adequate (Graham and Welch, 1996). However, there is evidence that the nutrient requirement of plants may be greater in the presence of pathogens (Webb and Graham, 1990).

To determine optimum conditions to investigate the interaction between differing wheat cultivars, Zn nutrition and *R. solani* infection severity, three preliminary experiments were conducted in a controlled environment with a combination of cultivars, soil types, levels of applied Zn and *R. solani* inoculum levels to determine which combination of variables is suitable for studying the interaction between *R. solani* infection and Zn nutrition.

3.2 General methods

Three experiments were conducted to determine optimum conditions for Zn × *Rhizoctonia* interactions. Table 3.1 outlines the design of the three experiments.

Inoculum source

Inoculum of *Rhizoctonia solani* Kühn (*R. solani*) (Anastomosis group 8 (AG 8) isolate RS21) was obtained from the Department of Soil Science, The University of Adelaide. Inoculum was in the form of colonised seeds of white millet (approximately 1.0 mm in diameter). The colonised seeds were stored at -20 °C to minimise premature growth of the fungus.

Cultivars

Seeds of the wheat cultivars, *Triticum aestivum* L. cvs Excalibur and Songlen, and *T. turgidum* conv. *durum* (Desf.) McKay cv Durati, were obtained from field trials where plants had been treated with two or more rates of Zn application. Seeds of each cultivar were further selected on the basis of seed Zn concentration (Table 3.2). Seeds with low Zn concentration were chosen because higher seed Zn concentration results in improved vegetative growth when plant-available Zn in soil is limited (Rengel and Graham, 1995).

Surface sterilisation of seed

Seeds were surface-sterilised in 70% (v/v) ethanol for 1 min, 1% (v/v) hypochlorite for 5 min and then rinsed three times with double de-ionised (DD) water (18 MΩ.cm resistivity). Seeds were soaked in aerated DD water overnight and germinated on moistened filter paper for 36 h at 20 °C before sowing.

Table 1.1. Description of the three experiments reported in this chapter.

Experiment	Title	Soil type	Zn levels (mg kg ⁻¹)	<i>R. solani</i> levels (propagules kg ⁻¹)	Inoculum source	Cultivars	Replicates
Experiment 1	Expression of Zn deficiency in wheat in two soil types	Laffer sand Mt. Compass sand	0 and 0.8	No propagules were applied	Nil	Excalibur Durati	2
Experiment 2	Selecting <i>R. solani</i> inoculum levels	Laffer	0, 0.04 and 0.4	0, 2, 4 and 8	Millet seed	Excalibur Durati	4
Experiment 3	Pot comparison	Laffer	0.05 and 0.2	16	Millet seed	Songlen	5

Table 3.2. Zinc concentration and content in the seeds of the three wheat cultivars used in the preliminary experiments. Seeds of each cultivar were sieved to a size between 2.5 and 3.0 mm. Values are means $\pm$ SE of five replicates each containing 15 seeds.

Cultivar	Zn concentration (mg kg ⁻¹)	Zn content (ng seed ⁻¹)
Excalibur	8.4 $\pm$ 0.2	280 $\pm$ 30
Songlen	11.1 $\pm$ 0.4	498 $\pm$ 10
Durati	10.0 $\pm$ 0.4	330 $\pm$ 40

Basal nutrients

Calcium carbonate (CaCO₃) was added to dry soil at 0.3 % (w/w) in order to increase soil pH and consequently reduce the availability of Zn in the soil. A complete nutrient solution (excluding Zn) was prepared with DD water and analytical grade chemicals, and applied to the soil. The final concentration of each compound in the soil was (mg kg⁻¹ of soil): NH₄NO₃ (350), KH₂PO₄ (150), K₂SO₄ (120), MgSO₄.7H₂O (90), MnSO₄.4H₂O (7), H₂MoO₄.H₂O (0.5), CoSO₄.7H₂O (1), NiSO₄.7H₂O (0.15), CuSO₄.5H₂O (10), H₃BO₃ (1), and FeSO₄.7H₂O (0.7).

Applied Zn was in the form of a solution of ZnSO₄.7H₂O added separately to each soil sample to produce the desired level of Zn. Table 3.1 lists the concentrations of Zn used in each experiment.

Soil types

Laffer sand

A Zn-deficient siliceous sand was collected from a naturally vegetated area near Tintinara (230 kilometres southeast of Adelaide) in South Australia, classified as Dy 5.43 (Northcote, 1971). It was leached of soluble salts in three changes of reverse osmosis (RO) water, organic matter particles were removed by flotation during the washing process, and the soil was air-dried before use. Laffer sand has a water holding (field) capacity of approximately 12%.

Mt. Compass sand

Mt. Compass sand is a podzolic soil from an area near Mt. Compass (40 kilometres south of Adelaide) in South Australia, classified as Uc 2.32 (Northcote, 1971). The sand was air-dried and not washed prior to experimentation because of the low organic matter content and in past experience it has been shown to contain low levels of known soluble salts. Mount Compass sand has a water holding capacity of approximately 18%.

Procedure

Air-dried soil (1 kg) was thoroughly mixed with basal nutrient solution before potting. Six hundred millilitre milk containers were lined with plastic bags and filled with 400 g of basal nutrient treated soil. Half the desired amount of inoculum (see Table 3.1 for amounts of inoculum applied) was placed on the soil surface and covered with a further 300 g of soil. The remaining half of inoculum was applied to the new soil surface and covered with a final 300 g of soil. Uninoculated treatments were potted in the same manner using inoculum that had been autoclaved for 1 hour at 121 °C and 100 kPa, on each of two consecutive days. To allow *R. solani* colonisation of soil, pots were watered to 10% moisture content (w/w) and incubated for 2 weeks prior to sowing in a controlled-environment chamber adjusted to the following conditions: photosynthetically active radiation at the pot level of $450 \mu\text{mol m}^{-2} \text{s}^{-1}$, 10/14 h light/dark and 15/10 °C day/night cycle.

Sowing and post-harvest

After incubation of soil, five pre-germinated seeds were planted in each pot and water was added to achieve 12% moisture (w/w) content for Laffer sand and 18% moisture content for Mt. Compass sand. The water content was maintained for the duration of each experiment by regular weighing and watering. After 7 days, seedlings were thinned to 3 per pot, and the soil surface was covered with acid-washed, black polypropylene beads to reduce water loss and algal growth.

At harvest, shoots were removed at the soil surface and dried in a hot-air oven at 65 °C for 48 h before dry weights were determined. Roots were washed free of soil with RO water and placed in plastic self-seal bags and stored at 4 °C. Dried shoots were digested according to Zarcinas (1984) for determination of nutrient concentrations by an Inductively Coupled Plasma Optical-Emission Spectrometer (ICP).

Statistical Analysis

Statistical analyses were performed using Super Anova (Abacus Concepts, Inc., California).

3.3 Experiment 1. Expression of Zn deficiency in wheat in two soil types

3.3.1 Aim

The aim of testing two soil types was to select an appropriate growth medium low in plant-available Zn. Other researchers studying Zn deficiency in pot experiments have used Laffer sand. However, the location of the collection site is 230 km from Adelaide. Mount Compass sand is more easily obtainable but had not been used previously to demonstrate Zn deficiency in pot studies.

3.3.2 Materials and methods

Experimental design

A factorial experiment combining two soil types, two rates of Zn applied to soil and two cultivars (Table 3.1, experiment 1) was conducted in pots under controlled conditions as described in Section 3.2. This gives a total of 8 treatments. Each treatment was duplicated. Plants were harvested 35 days after sowing.

3.3.3 Results

Visual Observations

The cultivars Excalibur and Durati displayed no visual symptoms of Zn deficiency when grown in Mt. Compass sand, regardless of the rate of Zn application. In contrast, plants grown in Laffer sand without added Zn were visibly smaller than the Zn-supplied plants 11 days after sowing. Symptoms of Zn deficiency were evident on oldest leaves of both cultivars 13 days after sowing when no external Zn was supplied. However, the application of 0.8 mg kg^{-1} Zn appeared to be adequate for normal growth, as plants displayed no visible symptoms of Zn deficiency.

Shoot dry weight

The shoot dry weight of plants grown in the various experimental conditions outlined are displayed in Figure 3.1. Application of 0.8 mg kg^{-1} Zn to plants grown in Mt. Compass sand gave no significant increase in shoot dry weight of either cultivar compared to the control with no added Zn. In contrast, the application of 0.8 mg kg^{-1} Zn to plants grown in Laffer sand significantly increased the dry weight of shoots of both Excalibur and Durati cultivars. It is evident that Excalibur and Durati achieve similar shoot dry weights when grown without Zn application in either soil type, although the shoot dry weight of both cultivars was depressed when grown in Laffer sand without applied Zn compared to Mt Compass sand without applied Zn. When Zn was supplied to soil, Durati attained greater shoot dry weight than Excalibur regardless of soil type, although this effect was more pronounced when plants were grown in Laffer sand.

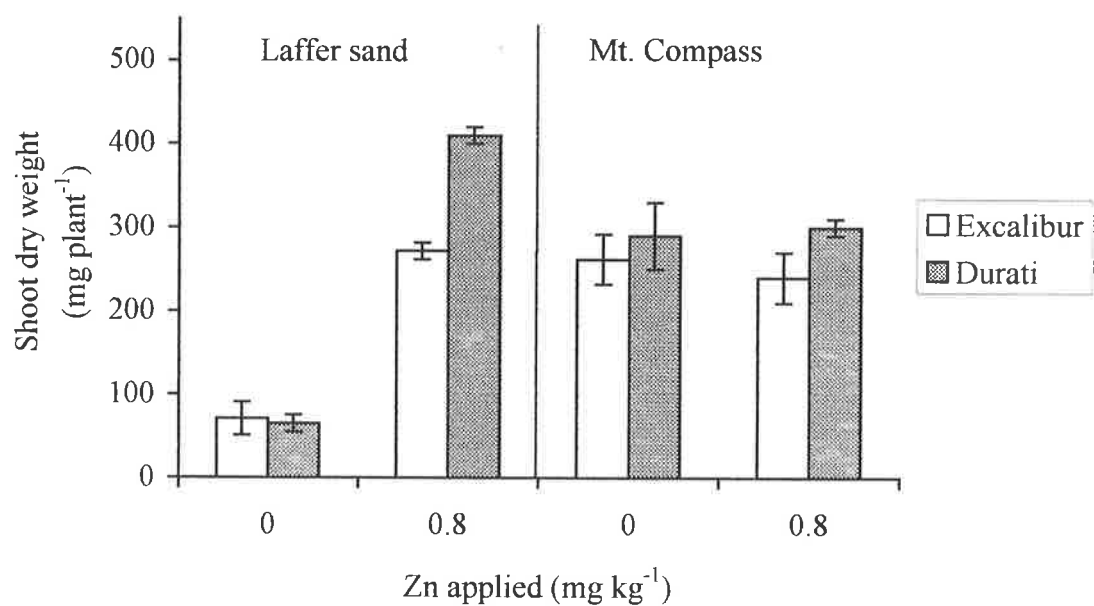


Figure 3.1. Shoot dry weight (mg plant⁻¹) 35 days after sowing of two wheat cultivars (Excalibur and Durati), grown with two levels of soil applied Zn in two soil types (Experiment 1). Values are means of duplicates; vertical bars indicate $\pm$ SE.

Zn concentration

When both cultivars were grown in Laffer sand without added Zn, Zn concentration of shoots of Durati was below the critical value of 16 - 20 mg kg⁻¹ (*cf.* Dang *et al.* 1993b; Reuter and Robinson 1997). Excalibur grown in Laffer sand with no added Zn had a higher Zn concentration in shoots than Durati grown under the same conditions, although this effect was not significant (Figure 3.2). However, the shoot Zn concentration of Excalibur appears to be marginal for optimal growth as defined above. The application of 0.8 mg kg⁻¹ Zn to plants of both cultivars grown in Laffer sand resulted in Zn concentration well above the critical value. When both Excalibur and Durati are grown in Mt. Compass sand without added Zn, shoot Zn concentration was above the critical level for optimal plant growth and that concentration was further enhanced in both cultivars when 0.8 mg kg⁻¹ Zn was applied.

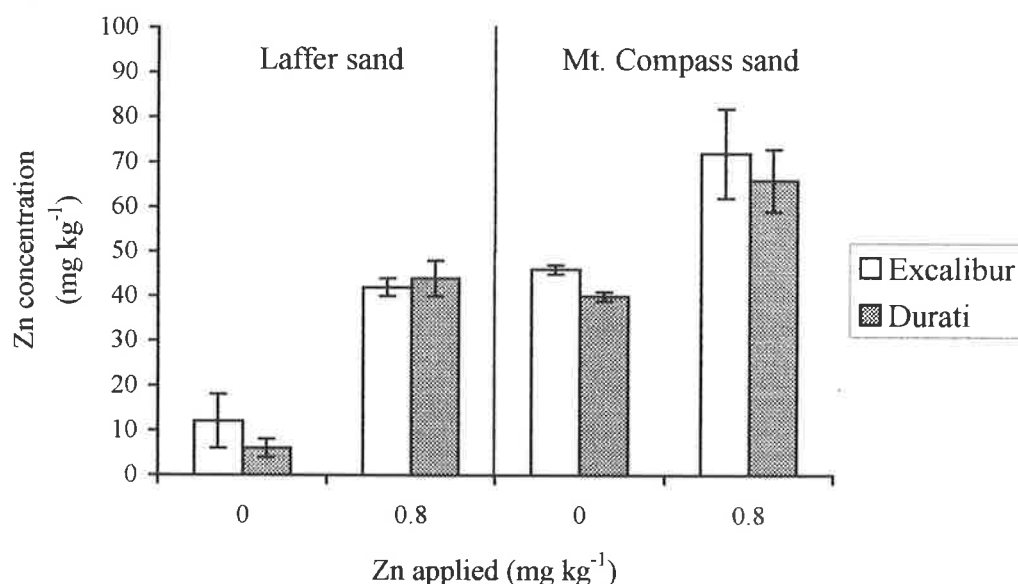


Figure 3.2. Zn concentration in shoots (mg kg⁻¹) 35 days after sowing of two wheat cultivars (Excalibur and Durati), grown with two levels of soil applied Zn (Experiment 3.1). Values are means of duplicates, vertical bars indicate ± SE.

Further analysis of concentrations of other nutrients (data not shown) in both cultivars grown in the two soil types with ($0.8 \text{ mg Zn kg}^{-1}$) and without (0 mg Zn kg^{-1}) Zn revealed copper (Cu) concentrations in shoots to be approximately 10 times higher (in the $0.8 \text{ mg Zn kg}^{-1}$ treatment) than the recommended critical level (Reuter and Robinson, 1997) for optimum growth of wheat. All other nutrients were within the recommended range (Reuter and Robinson, 1997).

3.3.4 Discussion

The growth of both Excalibur and Durati cultivars in Mt. Compass sand showed no visible symptoms of Zn deficiency regardless of whether Zn was applied or not. It was therefore not surprising that measurements of shoot Zn concentration demonstrated that plants of both cultivars had an adequate level of Zn for optimal growth when grown on Mt. Compass sand. In contrast, plants grown in Laffer sand without soil-applied Zn displayed Zn deficiency symptoms and were limited in yield of shoots compared to plants grown in Mt. Compass sand. This deficiency was overcome by the application of Zn. Shoot Zn concentration of both cultivars grown in Laffer sand without added Zn was below the critical value for optimum growth. Although the shoot Zn concentration of Excalibur grown in Laffer sand without Zn applied was determined to be marginally adequate for optimal plant growth, the visible Zn deficiency symptoms of Excalibur plants suggested that the plants were indeed deficient. Application of Zn to plants grown on Laffer sand increased shoot Zn concentration to a level above that required for optimal plant growth.

This experiment demonstrates that Laffer sand has a low availability of Zn to plants (Figure 3.1 and 3.2) that can be alleviated with the application of Zn. Laffer sand is therefore considered to be the ideal growth medium for investigations of Zn efficiency as both sub-optimal and optimal levels of Zn required for plant growth can be attained.

3.3.5 Conclusion

Laffer sand was selected as the most appropriate medium for further pot experimentation in the investigation of cultivar differences in the interaction between Zn and *R. solani*. The basal nutrient concentration of Cu was reduced from 10 mg CuSO₄ kg⁻¹ to 2 mg CuSO₄ kg⁻¹ in all further experiments as the concentration of Cu in whole shoots was high. The growth period was also reduced as plant roots were coiled around the bottom of the pot, which may impose a problem when *R. solani* is added to the system. If the root systems are too large, the analysis of *R. solani* infection may be too difficult to be carried out satisfactorily.

3.4 Experiment 2. Selecting levels of *R. solani* inoculum

3.4.1 Aim

To determine a level of *R. solani* inoculum that produces a level of infection, which reduces dry matter production in wheat without killing the plants.

3.4.2 Materials and methods

Experimental design

A complete factorial experiment combining three rates of soil-applied Zn, four levels of *R. solani* inoculum, and two wheat cultivars (as described in Table 3.1, experiment 2) was conducted in pots under controlled conditions as described previously. In total, 24 treatments were examined, and each treatment was replicated four times.

Procedure

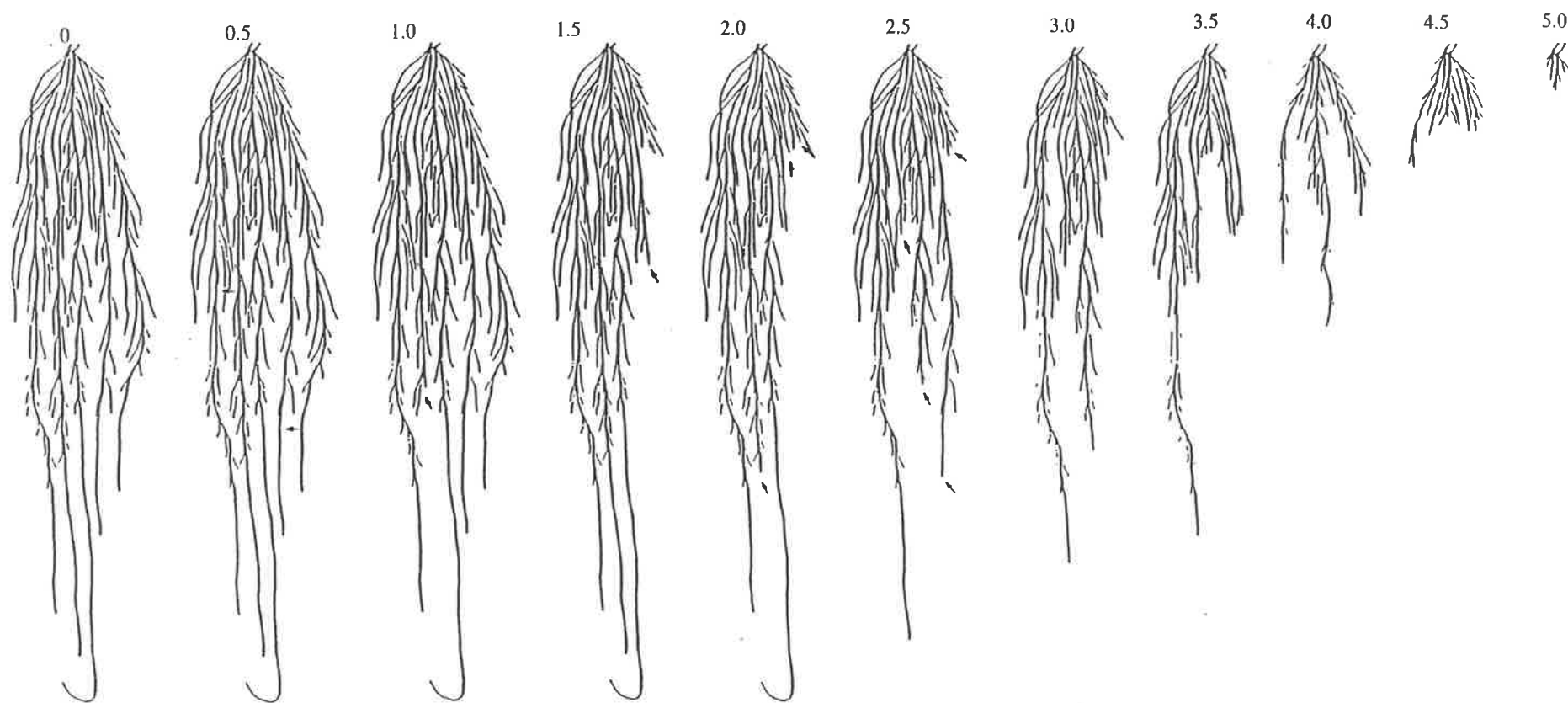
Basal nutrient concentrations, Zn applications and inoculum application were as described in Section 3.2 (General methods). However, Cu was decreased as suggested in Section 3.3.5. All treatments were imposed on plants grown in Laffer sand. Seven days

after sowing, seedlings were thinned to 4 plants per pot. Plants were harvested 28 days after sowing, and dry weight of roots and shoots was recorded.

Disease scoring method

Roots were visually scored for disease severity, using the root rot scale reported by Sweetingham and MacNish (1992) (Figure 3.3). The disease score ranges from 0 to 5 in increments of 0.5 and is inversely related to the root length of seminal roots. Therefore, the root rot scale is based on growth of roots and evidence of lesions. Scores of 0 and 0.5 have the same length of root, but 0.5 has clear evidence of lesions (Figure 3.3).

Figure 3.3. Root rot scale for disease scoring system developed by Sweetingham and MacNish (1992) at half actual size. The disease score steps in 0.5 increments, inversely related to the root length of seminal axes. Arrows indicate points of spear tips or lesions.



3.4.3 Results

Plant growth

Figure 3.4 outlines the relationship between applied Zn and level of *R. solani* inoculum for cultivars Excalibur and Durati. Statistical analysis of this data set reveals all treatment interactions are not significant at $\alpha = 0.05$, only main effects of cultivar and Zn application were significant. Keeping this in mind however, the general tendencies of the data can be discussed. Both cultivars grown in soil with 0.4 mg kg^{-1} of applied Zn produced more shoot dry matter than plants grown in soil without applied Zn ($P < 0.001$ for the effect of Zn). The application of 0.04 mg kg^{-1} Zn to the control had a more pronounced effect on shoot dry weight in Excalibur (29%) than in Durati (7%). For both cultivars grown in soil without added Zn, a decrease in shoot dry weight was evident with the addition of 8 propagules of *R. solani*, although the difference was not significant. However, the statistical analysis of this data set takes into account the trend over all treatments. As can be ascertained from Figure 3.4, the trend of decreasing shoot dry weight with increasing *R. solani* inoculation was not apparent in either cultivar when 0.04 mg kg^{-1} Zn was added but was observed only in Excalibur supplied with 0.4 mg kg^{-1} Zn. Infection by *R. solani* significantly reduced shoot yield in Excalibur when four or eight propagules of inoculum were supplied in soil supplemented with 0.4 mg kg^{-1} Zn. Shoot dry matter production was not significantly reduced in Durati when infected by either level of inoculum in soil supplemented with 0.4 mg kg^{-1} Zn. Indeed, infection of Durati with four propagules of *R. solani* appeared to increase shoot yield when the plants were supplemented with 0.4 mg kg^{-1} Zn. Both Excalibur and Durati had similar shoot dry weight when grown with 0.4 mg kg^{-1} Zn and eight propagules of inoculum.

Zn concentration

Both Excalibur and Durati supplied with 0 and $0.04 \text{ mg Zn kg}^{-1}$ soil were severely deficient in Zn (Figure 3.5). Inoculation of plants grown in these Zn-deficient conditions did not significantly decrease shoot Zn concentration in either cultivar over any

of the inoculation densities studied. Application of $0.4 \text{ mg Zn kg}^{-1}$ soil increased the shoot Zn concentration of both Excalibur and Durati above the level required for optimal plant growth ($16 - 20 \text{ mg Zn kg}^{-1}$ soil respectively; Dang *et al.* 1993b; Reuter and Robinson 1997) regardless of *R. solani* infection (Figure 3.5).

Cu concentration

The decrease of the rate of soil application of CuSO_4 to 2 mg kg^{-1} based on results from the previous experiments resulted in Cu concentrations within the recommended range for optimum growth of wheat (data not shown) (Reuter and Robinson, 1997).

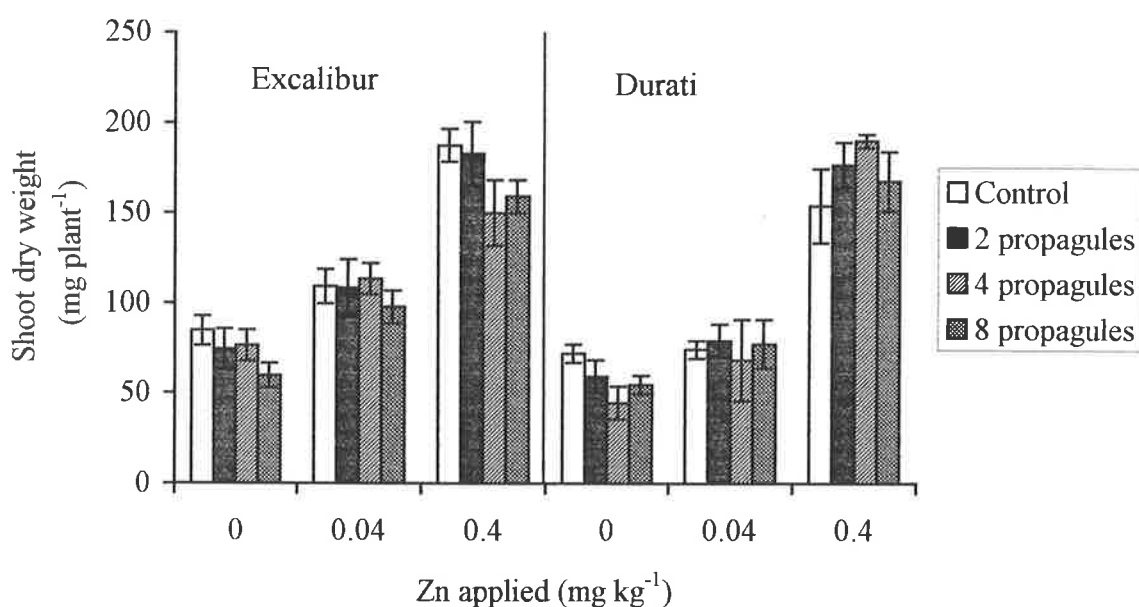


Figure 3.4. Shoot dry weight (mg plant^{-1}) of two wheat cultivars (Excalibur and Durati) grown at three levels of soil Zn and four levels of *R. solani* inoculum (Experiment 2) for 28 days. Values are means $\pm$ SE of three replicates.

Probabilities of treatments significantly influencing shoot dry weight: cultivar, $P = 0.01$; zinc, $P = 0.001$; Rhizoctonia, $P = 0.41$. All treatment interactions were not significant at $\alpha = 0.05$.

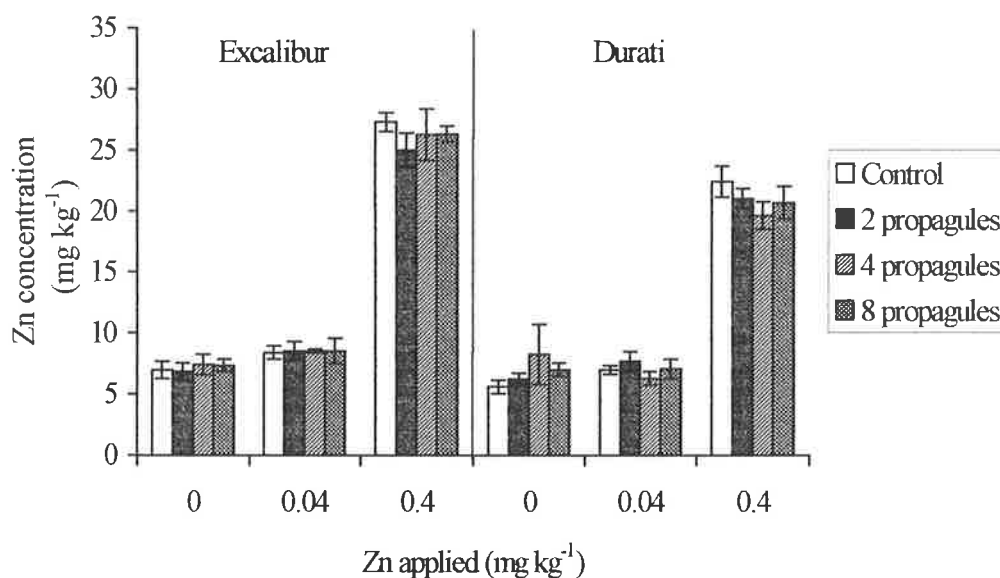


Figure 3.5. Zn concentration (mg kg^{-1}) in shoots of two wheat cultivars (Excalibur and Durati) grown with three levels of soil Zn and four levels of *R. solani* inoculum (Experiment 2) for 28 days. Values are means of three replicates $\pm$ SE.

Probabilities of treatments significantly influencing Zn concentration: cultivar, $P = 0.000$; zinc, $P = 0.0001$; Rhizoctonia, $P = 0.82$; cultivar $\times$ zinc, $P = 0.0001$. All other treatment interactions were not significant at $\alpha = 0.05$ level.

Disease score of root rot caused by R. solani

There was no significant difference between cultivars in susceptibility to infection by *R. solani*. Data from both cultivars infected with differing inoculation of *R. solani* were therefore pooled. Similarly, no significant difference was noted between cultivars in relation to susceptibility to infection over the range of Zn concentrations applied, this data was therefore also pooled. As is evident in Table 3.3, the disease score of plants infected by *R. solani* was not significantly altered by increasing concentrations of soil applied Zn. However, higher rates of *R. solani* inoculum generally caused more damage to the roots (Table 3.3).

Table 3.3. Disease score of wheat roots infected by *R. solani* (Experiment 2) and harvested 28 days after sowing. Disease score was measured on 0 to 5 scale as described in Figure 3.3. Data were pooled across cultivar and soil Zn treatments because main effects and all interactions involving these main effects were not significant. Means followed by the same letter in each row are not significantly different according to Fishers protected LSD (significance level $P \leq 0.05$).

<i>R. solani</i> treatment				
	propagules kg ⁻¹ soil			
	0	2	4	8
Disease score	0.11 a	1.69 b	2.66 c	3.07 c
Zn treatment				
	mg Zn kg ⁻¹ soil			
	0	0.04	0.4	
Disease score	1.91 a	1.79 a	1.87 a	

3.4.4 Discussion

Thongbai *et al.* (1993b) have reported a suppression of *R. solani* disease severity on wheat with the addition of 0.04 and 0.1 mg Zn kg⁻¹. This experiment did not display a noticeable decrease in disease severity when 0.04 mg kg⁻¹ Zn was added to the soil. This may be due to differences in soil collection and preparation, wheat cultivars used or environmental conditions. Thongbai *et al.* (1993b) grew plants at 15/10 °C day night cycle for 50 days after sowing compared with the 28 days in the present study. Plants in the present study could not have been grown for a period of 50 days as the Zn deficiency expressed in Zn-limiting conditions would have been lethal. This may suggest that 0.04 mg Zn was not high enough as a marginal zinc treatment. The light intensity of the growth chamber was not specified in the published data of Thongbai *et al.* (1993b). A comparison between the present study and that of Thongbai *et al.* (1993b) is limited in scope. In Bean (*Phaseolus vulgaris*) it has been shown that with increasing light intensity plant dry weight increases in Zn-sufficient plants but not in zinc-deficient plants (Marschner, 1995). As Thongbai *et al.* (1993b) solely used the growth chamber it is impossible to know what intensity and type of lighting and what age the bulbs were at the time of previous experimentation.

Under the experimental conditions of the present study it has been determined that the interaction between *R. solani* inoculum density, concentration of applied Zn and cultivar is not significant at the 95 % confidence interval either in terms of shoot dry

weight produced or concentration of Zn in the shoots. However, given that the statistical analysis was performed on the trend apparent over three soil Zn concentrations it is possible to make comparisons between single variables when all other variables are kept constant, i.e. comparisons between Excalibur plants grown in soil with $0.4 \text{ mg Zn kg}^{-1}$ and differing inoculum densities can be made. Therefore the most important observations from this preliminary experiment are:

1. For plants of both cultivars grown on soil with 0.04 mg kg^{-1} applied Zn, no significant effect of increasing inoculation density can be observed when measuring either shoot dry matter or shoot Zn concentration.

2. Whilst there is no difference between cultivars in disease score over the range of Zn concentrations applied or with differing densities of inoculum application, an increase in disease score is apparent in both cultivars when increasing densities of inoculum are used. The downward trend of shoot dry weight with increasing *R. solani* inoculum in 4 out of 6 cases in Figure 3.4, while not significant, stands in strong contrast with the non-effect of *R. solani* inoculum on Zn concentration. It is apparent that the experimental conditions applied here differ to those of Thongbai *et al.* (1993b).

3.4.5 Conclusion

Durati was used, in experiment 1 and 2, because of its extreme sensitivity to Zn in this exploratory experiment and it is clear that the system is adequate, in that both Durati and Excalibur showed symptoms of Zn deficiency. In subsequent chapters, it was felt desirable to compare bread wheats with bread wheats rather than including durum wheats, so Songlen, Gatcher and Excalibur, although not as sensitive as Durati, were used.

The maximum concentration of applied Zn determined to be necessary to give adequate growth and a significant difference to the control was 0.4 mg kg^{-1} .

The increase in inoculum from 2 to 4 propagules demonstrated no significant difference in disease score, however, there was no significant difference in disease scores of Excalibur plants when inoculated with 4 or 8 propagules of *R. solani*-colonised millet

seed. This suggests the hyphae of *R. solani* may not be exploring the soil to the maximum extent or there is not enough inoculum in the system. A plausible explanation for the lack of differences in disease scores may be the type of pot used in this study. Pots used were cartons, which have flexible sides, compared with the rigid pots used by Thongbai *et al.* (1993b). It is possible that movement of the sides of the pots during weighing and watering may have disturbed established hyphae of *R. solani* in the soil resulting in a consequent reduction in fungal vigour. Owing to the low level of infection observed in the previous experiment and the lack of difference in root damage between 4 and 8 propagules a third experiment was performed using 16 propagules of *R. solani*. The following experiment was conducted in pots that had flexible sides (like experiment 2) or were rigid, in order to maximise *R. solani* infection.

3.5. Experiment 3. Pot comparison

3.5.1 Aim

To determine infection levels caused by *R. solani* on wheat in two different pot types. It is hypothesised from the previous experiment that movement of the sides of the pots during weighing and watering may shear the soil and consequently break the hyphae of *R. solani* and reduce the vigour of the fungus. The wheat cultivar used for this experiment was Songlen, which was inoculated with 16 propagules of *R. solani*. These conditions most closely represent those of Thongbai *et al.* (1993b) and allow a more accurate assessment of the effect of pot type on infection capability of the fungus.

3.5.2 Materials and methods

Experimental design

A factorial experiment combining two rates of soil-applied Zn, two types of pots and one cultivar (cv. Songlen) (Table 3.1, experiment 3) was conducted under controlled conditions as described earlier (Section 3.2). Songlen was used in this experiment to enable a direct comparison with experiments conducted by Thongbai *et al.*, (1993b). All

pots were inoculated with 16 propagules of *R. solani*. The number of propagules was increased due to the low level of infection observed in section 3.4. Zinc levels were chosen on the basis of the results presented in section 3.4; the lower level of applied zinc was raised to 0.05 mg Zn kg⁻¹ to determine if this still produced Zn-deficient plants (and was high enough as a marginal Zn treatment) and the higher level was adjusted to 0.2 mg Zn kg⁻¹ to determine if this level resulted in Zn-adequate plants. A total of four treatments were examined with each treatment being replicated five times.

Pot type

The pots used were milk cartons as used previously and milk cartons held rigid in a hard plastic holder with handle. The use of plastic holders with handles meant that cartons could be easily moved for weighing and watering without causing disturbance of soil.

3.5.3 Results

The shoot dry weight, Zn concentration and level of *R. solani* infection of wheat plants were not significantly affected by pot type (Table 3.4). The application of Zn increased both the shoot dry weight and Zn concentration; however, there was no significant decrease in disease score (Table 3.4).

Table 3.4. Shoot dry weight, Zn concentration and disease score of wheat plants (Songlen) 28 days after sowing in two different types of pots with two Zn application rates (Experiment 3). Values are means $\pm$ SE of five replicates. No treatments were significantly different at $\alpha = 0.05$ level.

Zn treatment (mg kg ⁻¹ soil)	Container	Shoot dry weight (mg plant ⁻¹)	Zn concentration (mg kg ⁻¹)	Disease score
0.05	Solid	193 $\pm$ 33	12.4 $\pm$ 1.3	1.7 $\pm$ 0.3
	Flexible	180 $\pm$ 17	12.2 $\pm$ 1.9	1.8 $\pm$ 0.5
0.2	Solid	273 $\pm$ 37	21.1 $\pm$ 1.0	1.6 $\pm$ 0.4
	Flexible	263 $\pm$ 17	21.6 $\pm$ 1.2	2.0 $\pm$ 0.2

3.5.4 Discussion

The similar levels of infection in plants caused by *R. solani* in both types of pots does not support the hypothesis that handling of the flexible pots disturbed the soil and hence the hyphae of the fungus. The choice of flexible cartons for pots does not explain the difference in results of the current experiment with results of Thongbai *et al.* (1993b). The application of Zn did not suppress *R. solani* infection in either pot type.

3.5.5 Conclusion

There is no advantage in using solid pots to increase the levels of infection caused by *R. solani*, therefore the inexpensive flexible pots were used for further experiments together with 16 propagules of inoculum. It is concluded that extreme action would have to be applied to the pots to have any negative consequence on growth. This experiment was also unable to confirm the results of Thongbai *et al.*, (1993b) on the suppression of *R. solani* infection with applied Zn.

3.6 General Discussion

The preliminary experiments in this chapter have not only allowed for a system of experimentation to be established they have also given some information on the Zn status of wheat and the susceptibility to infection by *R. solani*.

Laffer sand was found to be the most appropriate soil for experimentation involving Zn deficiency (Experiment 1) and in subsequent experiments it was also proven to be an adequate growth medium for *R. solani*. Colonisation of the soil by *R. solani* is difficult to assess but damage to plant roots (disease score) indicated no significant difference between the application of 4 and 8 propagules of fungus (Experiment 2; Table 1.4). Experiment 3 utilised 16 propagules of *R. solani*. When one compares experiment 2 (Table 3.3) and 3 (Table 3.4) it is noted that the disease score does not increase in proportion to the number of applied propagules. It is difficult to make comparisons over separate experiments but it is possible that disease score is not the most appropriate technique to assess *R. solani* infection, particularly over differing nutrient regimes. Chapter 5 will address these problems. Experiment 2 has also demonstrated that there is

no advantage in using solid pots to increase the levels of infection caused by *R. solani*, therefore subsequent chapters utilised the inexpensive flexible pots.

Many researchers have hypothesised that nutrient deficiency predisposes plants to infection by pathogens (Sparrow and Graham, 1988; Graham and Welch, 1994; Grewal *et al.*, 1996). In the case of Zn nutrition and pathogen attack, it has been demonstrated that susceptibility to pathogens is accelerated under Zn deficiency (Thongbai *et al.*, 1993b; Grewal *et al.*, 1996). The data presented here does not support such a claim and further study is required.

In Experiment 2 (Section 3.4.3) Excalibur displayed a decrease in yield caused by *R. solani* when Zn was sufficient for growth (Figure 3.4). As demonstrated in Figure 3.5, Zn deficiency does not appear to be the cause of this yield loss. However, since *R. solani* is a macerative pathogen infecting plants early in the seedling stage, high Zn nutritional status in the seedling due to high Zn seed content (Rengel and Graham, 1995) may be a means for protecting seedlings from *Rhizoctonia* root rot. As low Zn seed was used for preliminary experiments, the lack of effect of Zn applied at 0.04 mg kg^{-1} may be explained by the possibility that the seedlings' root systems were not large enough (either through being inherently shorter or being restricted by *R. solani* infection) to provide adequate uptake of Zn. When higher concentrations of Zn were added, this phenomenon was no longer noticeable as the amount of Zn in the environment was so high that even seedlings with inefficient uptake could maintain an adequate level of Zn uptake for growth. To study this further it is required that the effect of high and low seed Zn concentration on both Zn uptake and *R. solani* infection be further investigated.

Chapter 4

The effect of Zn source on the growth of wheat infected by *Rhizoctonia solani* root rot

4.1 Introduction

It is hypothesised that the concentration of Zn in the developing plant is an important aspect of disease resistance and/or tolerance. Increased Zn concentration in the shoots of developing wheat plants may result from higher Zn content of the sown seed, high levels of applied Zn to the soil and also the ability of the plant to efficiently take up Zn from soil.

Seed size and higher seed Zn content are important determinants of seedling vigour and early growth of cereals (TeKrony and Egli, 1991) and may also be important in increasing resistance to various pathogens (Graham and Webb, 1991). Wheat plants grown from seed with high Mn concentration have been demonstrated to display increased tolerance to take-all (Wilhelm, 1992; McCay-Buis *et al.*, 1995). In addition, seed soaked in micronutrient solution prior to germination, produced plants with better germination rates and increased resistance to *Fusarium* infection (Sadasivan, 1965).

Seed nutrient reserves are important for the early establishment of crops in low-fertility soils (Singh and Bharti, 1985; Marcar and Graham, 1986, 1987; Longnecker *et al.*, 1988, 1991; Rengel and Graham, 1995). Longnecker *et al.* (1988) demonstrated that barley plants derived from high manganese (Mn) seed and grown in Mn-deficient soil had improved dry matter production in comparison to plants originating from low-Mn seed. It has also been shown that, during the first 3 weeks of development, wheat plants grown from seed with a high-Zn content produce more roots and shoots compared with those grown from low-Zn seed (Rengel and Graham, 1995). This indicates the importance of seed Zn content in early seedling establishment.

Increased seed nutrient reserves may reduce susceptibility of seedlings to pathogens (Graham and Webb, 1991; Rengel and Graham, 1995; McCay-Buis *et al.*, 1995). This is especially evident in the interaction between Mn nutrition and take-all infection of wheat. Under moderately-severe to severe take-all disease conditions, wheat plants germinating from seed with high Mn content were more vigorous, had an average of 11% less take-all (*Gaeumannomyces graminis* var *tritici*), and yielded an average of 165 kg ha⁻¹ more grain than plants germinating from seed of the same cultivar but with a lower Mn content (McCay-Buis *et al.*, 1995). Given the effect of Mn content on the severity of take-all infection, it is possible that other interactions between micronutrients and disease occur. Graham and Welch (1996) proposed that pathogens manage to infect weaker, Zn-deficient seedlings at the vulnerable emergence stage, and continue to retard seedling growth.

The following experiment is designed to investigate if high seed Zn content decreases infection by *R. solani*.

4.1.1 Aim

To determine the effect of soil-applied Zn and seed Zn content on the severity of *R. solani* infection in wheat cultivars differing in efficiency of Zn uptake from soil.

4.2 Materials and methods

Experimental design and treatment

A factorial experiment was conducted with two levels of applied Zn (0 and 0.4 mg Zn kg⁻¹ soil) together with two wheat cultivars with differing Zn efficiencies, and two levels of *R. solani* inoculum (control and 16 propagules kg⁻¹ soil). The cultivars were grown from seed with either a high or low Zn content (see Table 4.1). Each treatment was replicated four times. A total of 16 treatments were examined. Statistical analyses were performed using Super Anova (Abacus Concepts, Inc., California).

Soil and basal nutrients

A complete nutrient solution (excluding Zn) was prepared with DD water (18 M Ω .cm resistivity) and analytical grade chemicals as described in Chapter 3.

Inoculum

Inoculum preparation followed a modification of the method of McDonald and Rovira (1985). Seeds of white millet, approximately 1 mm in diameter, were placed in 250 ml flasks and soaked in DD water overnight, drained, then autoclaved for 1 hour at 121 °C and 100 kPa twice. Following each autoclave treatment, the flasks were shaken to prevent packing of the seed. *Rhizoctonia solani* (anastomosis group 8) cultures grown on potato-dextrose agar were cut into 1 cm³ plaques. Eight plaques were added to each flask of sterilised millet seed (250 g), and incubated at 25 °C for 3 weeks to allow complete colonisation of the seed. Flasks were shaken by hand weekly to achieve even colonisation of all seed. The inoculum was laid out aseptically on sterile filter paper in a laminar flow unit for approximately 8 hours to dry. The inoculum was then placed in airtight containers and stored at -20 °C.

Procedure

Growth conditions, Zn application and *R. solani* inoculum were as described in Chapter 3, Section 3.2.

Cultivars

The wheat cultivars Gatcher, considered to be a Zn-inefficient cultivar (Rengel and Graham, 1995), and Excalibur, considered to be Zn-efficient in field trials where Zn supply is limiting (Graham *et al.*, 1992), were used for these experiments. In this case a Zn-efficient cultivar is defined as a cultivar which takes up more Zn and produces more dry matter and grain yield in Zn-deficient conditions than another cultivar subject to the same conditions. All seeds were obtained from a field experiment near Lameroo (South

Australia) conducted in 1988 (Graham *et al.*, 1992). Seed with high and low Zn contents (see Table 4.1) were obtained from plants fertilised and not fertilised with Zn, respectively. Zinc was applied to plots as zinc oxysulphate (~30% Zn) with the seed and was supplemented with a foliar spray of ZnSO₄ at tillering (equivalent to 200 g Zn ha⁻¹; Graham *et al.*, 1992). The difference in seed Zn content between low-Zn and high-Zn seed was almost 3-fold (Table 4.1). The low-Zn seed of both genotypes had similar Zn content, while Gatcher had slightly higher seed Zn content than Excalibur for the high-Zn seed (Table 4.1). However, their analysis of variance did not display significant differences for the cultivar effect nor for the cultivar × Zn content interaction (Rengel and Graham, 1995). Therefore the two cultivars are as similar as practicable.

Table 4.1. Zinc concentration and content (Rengel and Graham, 1995) in the seeds of two wheat cultivars used in the present study. Seed was sieved to a uniform seed size (2.5-3.0 mm). Values are means ± SE of five replicates each containing 15 seeds given by Rengel and Graham (1995).

Cultivar	Zn status	Seed Zn	
		mg kg ⁻¹	ng seed ⁻¹
Excalibur	low Zn	8.4 ± 0.1	242 ± 5
Excalibur	high Zn	24.3 ± 0.3	660 ± 16
Gatcher	low Zn	9.5 ± 0.2	258 ± 18
Gatcher	high Zn	27.3 ± 1.2	743 ± 36

Prior to sowing, seeds were surface-sterilised in 70% (v/v) ethanol for 1 min, 1% (v/v) hypochlorite for 5 min and rinsed three times with DD water. Seeds were soaked in aerated, DD water overnight and germinated on moistened filter paper (Whatman No. 1) for 36 hours at 20 °C.

Sowing and post-harvest

Five pre-germinated seeds were sown in each pot to a depth of 1.5 cm and water was added to reach field capacity (12% moisture content (w/w)). The soil surface was covered with acid-washed, black polypropylene beads to reduce water loss and algal

growth. After 7 days, seedlings were thinned to 4 plants per pot. The experimental pots were weighed and watered to field capacity regularly, plants were grown in the growth chamber for 28 days. Temperature and light conditions were identical to those described in Section 3.2. On harvest shoot dry weight was recorded and nutrient status determined by ICP spectrometry as previously described in Section 1.2. Soil was washed from roots (in DD water) and the roots stored in sealed plastic bags at 4 °C. Roots were scored for disease severity using the root rot scale as described previously (see Figure 1.1), dried at 65 °C for 48 hours and weighed to determine root dry matter production.

4.3 Results

Shoot growth

Figure 4.1 outlines the relationship between seed Zn content, applied Zn and *R. solani* infection for the cultivars Excalibur and Gatcher. When comparing the shoot dry matter produced by the two cultivars, it is important to remember that the actual production of shoot dry matter is likely to be a genotypic effect inherent to the cultivar, however, several comparisons can be made. Shoot growth of Excalibur and Gatcher plants increased with the addition of Zn to soil regardless of seed Zn content (Zn treatment, $P = 0.0001$). Seedlings germinated from seeds with a high Zn content generally had a higher shoot dry weight, with the exception of Gatcher seedlings grown at 0.4 mg kg⁻¹ applied Zn (seed Zn content, $P = 0.0001$). Gatcher produced more shoot dry weight than Excalibur when additional Zn was present (either as high seed Zn or 0.4 mg Zn kg⁻¹ soil; Figure 4.1). The Zn-efficiency rankings [(Shoot dry weight of plants grown in Zn-deficient soil/Shoot dry weight of plants grown in Zn-sufficient soil) * 100] of Excalibur and Gatcher grown from low-Zn seed were 74 % and 44 %, respectively. Despite this, the application of Zn to Gatcher seedlings germinating from low-Zn seed gave a greater difference in shoot dry weight compared to the application of Zn to Excalibur seedlings germinated from low-Zn seed (cultivar effect, $P = 0.0001$). Indeed, the amount of shoot dry matter produced in Gatcher seedlings was not significantly different when 0.4 mg kg⁻¹ Zn was applied irrespective of whether the seedlings developed from seed with a high or low Zn content. In contrast, the application of 0.4 mg kg⁻¹ Zn to Excalibur seedlings demonstrates that

seedlings derived from a high-Zn seed maintain an increased shoot dry matter production compared to those derived from low-Zn seed.

When comparing the relative reduction in shoot dry weight of seedlings inoculated with *R. solani*, it is evident that plants infected by *R. solani* had lower shoot production than uninfected plants in both Zn treatments irrespective of seed Zn content and cultivar (*Rhizoctonia* effect, $P = 0.0001$). However, Excalibur displays a lower percentage reduction of dry matter when infected with *R. solani* regardless of Zn application or seed Zn content compared to Gatcher. This is evident from the observation that Excalibur plants, grown in a Zn-limiting environment, displayed a 51% *Rhizoctonia*-induced reduction in shoot dry weight, compared to a 46% reduction in shoot dry weight when grown in Zn-treated soil. However, Gatcher grown in a Zn-limiting environment, displayed a 55% *Rhizoctonia*-induced reduction in shoot dry weight, compared to a 59% reduction in shoot dry weight when grown in Zn-treated soil. It is interesting to note for both soil Zn applications the addition of *R. solani* reduced shoot dry matter of both cultivars to a similar level regardless of seed Zn content.

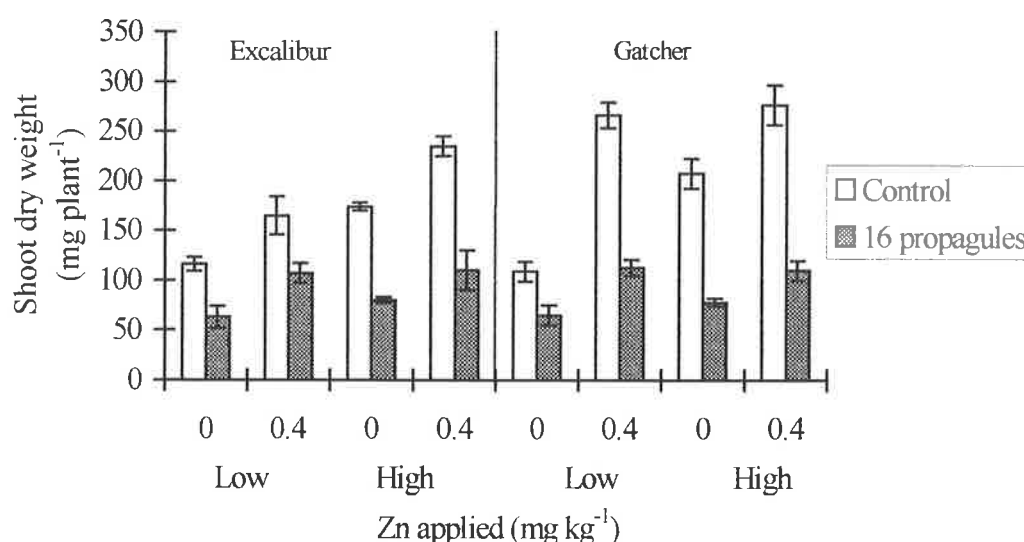


Figure 4.1. Shoot dry weight of two wheat cultivars (Excalibur and Gatcher) 28 days after sowing as influenced by seed Zn content (low, high), soil Zn application (0, 0.4) and *Rhizoctonia solani* inoculum (0, 16 propagules). Values are means $\pm$ SE of four replicates.

Probabilities of treatments significantly influencing shoot dry weight: cultivar, seed Zn, Zn treatment and *Rhizoctonia*, $P = 0.0001$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

Root growth

Two significant observations can be made on the interaction of treatments on seed content, Zn application and *R. solani* inoculum with respect to root dry weight (Figure 4.2). A significant decrease was observed in root dry weight caused by *R. solani* inoculum of Excalibur seedlings germinated from high Zn seed when no external Zn was applied. A second reduction in root dry weight caused by *R. solani* inoculum was observed in Gatcher plants grown from low Zn content seed when no external Zn was applied. ANOVA analysis demonstrates a significant difference between cultivars in relation to root dry weight production (main effect, $P = 0.0002$).

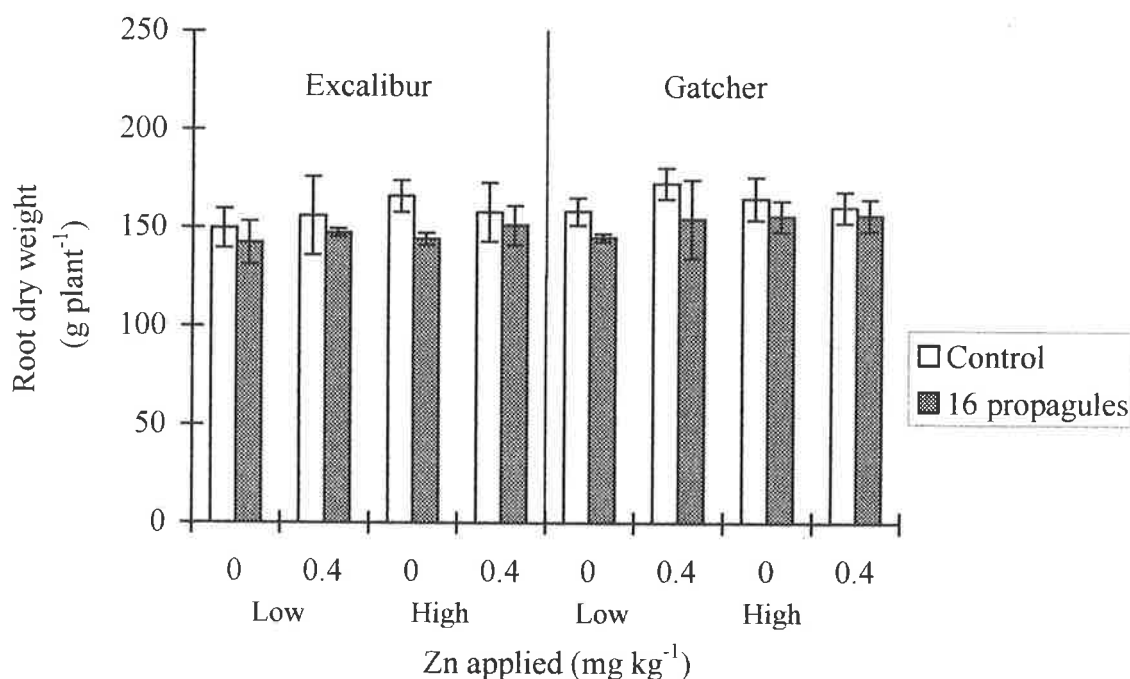


Figure 4.2. Root dry weight of two wheat cultivars 28 days after sowing grown with 0 or 0.4 mg kg⁻¹ soil applied Zn; with control or 16 propagules of *R. solani* inoculum. Values are means $\pm$ SE of four replicates.

Probabilities of treatments significantly influencing root dry weight: cultivar, $P = 0.0002$. All other main effects were not significant at $\alpha = 0.05$ level. All interactions were not significant at $\alpha = 0.05$ level.

The root/shoot ratio of plants is given as this parameter indicates changes in allocation of carbohydrates between roots and shoots. The root/shoot ratio of plants grown without soil-applied Zn is greater than that of plants grown in soil with applied Zn. In

addition, seedlings germinated from low-Zn seed tended to display higher root/shoot ratios than those germinated from high-Zn seed regardless of the application of soil Zn. Application of *R. solani* inoculum to soil increased the root/shoot ratio of both cultivars in all treatments (Figure 4.3).

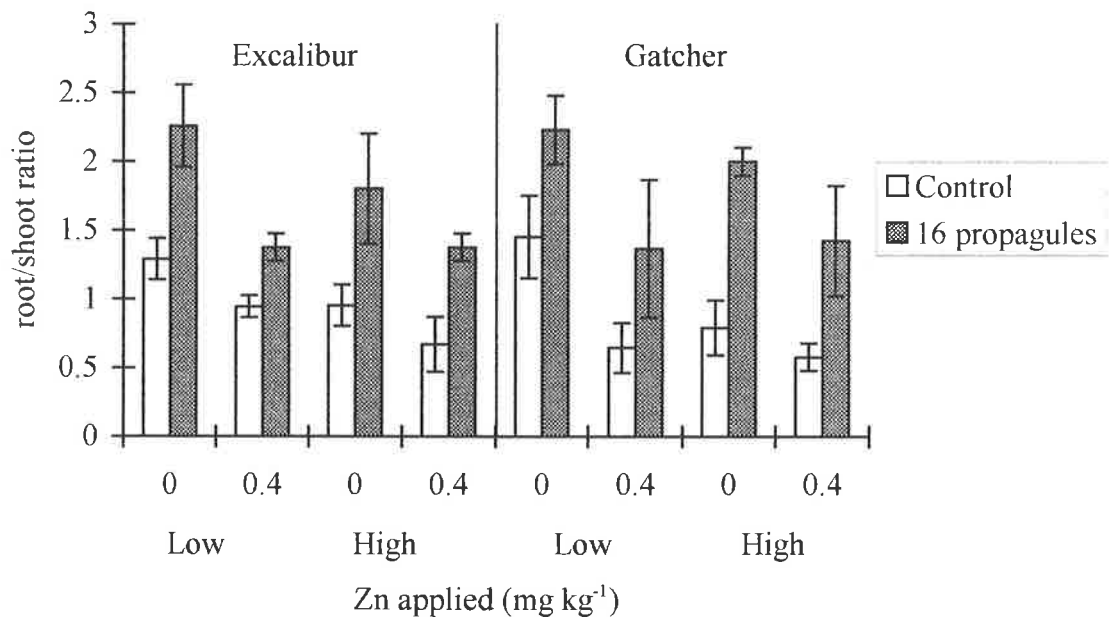


Figure 4.3. Root/shoot ratio of two wheat cultivars (Excalibur and Gatcher), grown with 0 and 0.4 mg Zn kg⁻¹ soil applied Zn; with control and 16 propagules of *R. solani* inoculum. Values are means of four replicates $\pm$ SE.

Zn concentration

The addition of Zn to soil greatly increased Zn concentration of shoots of both cultivars regardless of *R. solani* treatment (Figure 4.4) ($P = 0.0001$). Addition of *R. solani* inoculum decreased the shoot Zn concentration of both cultivars regardless of Zn application to soil or whether plants were grown from high or low Zn seed ($P = 0.0001$). All plants grown without soil-applied Zn had extremely low Zn concentrations of shoots below critical levels. A significant effect of seed content on shoot Zn concentration is evident from the analysis of variance ($P = 0.003$) and is most pronounced when no Zn was applied to soil. The interactions of seed Zn content, Zn treatment and *R. solani* inoculation were significant ($P = 0.019$) as were Zn treatment and *R. solani* inoculation ($P = 0.0001$).

The latter is most pronounced when observing the differences in shoot Zn concentration of plants grown with and without added Zn regardless of cultivar or seed Zn content.

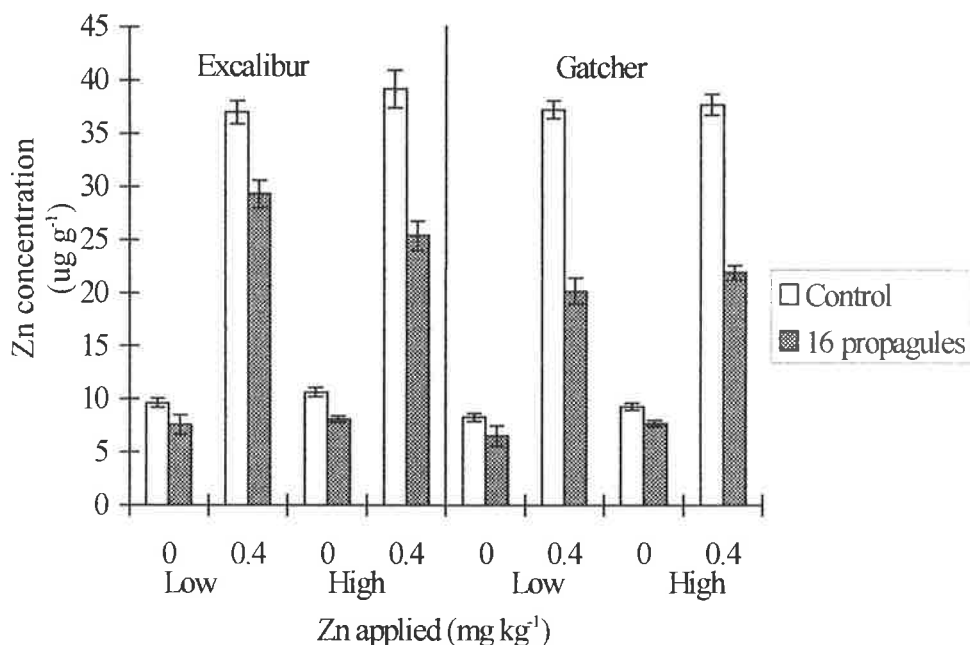


Figure 4.4. Zn concentration in shoots of two cultivars of wheat (Excalibur and Gatcher) 28 days after sowing, grown with 0 or 0.4 mg Zn kg⁻¹ soil applied or with control and 16 propagules *R. solani* inoculum. Values are means $\pm$ SE of four replicates.

Probabilities of treatments significantly influencing Zn concentration: cultivar, $P = 0.077$; Seed Zn, $P = 0.0003$; Zn treatment and Rhizoctonia, $P = 0.0001$.

Probabilities of interactions of treatments significantly influencing Zn concentration: Seed Zn $\times$ Zn treatment $\times$ Rhizoctonia, $P = 0.0190$; Zn treatment $\times$ Rhizoctonia, $P = 0.0001$.

Infection by Rhizoctonia solani

There was no significant difference in root infection by *R. solani* for the individual treatments or their interactions. Although it is possible that the application of 0.4 mg of Zn per kg soil to Excalibur grown from low-Zn seed decreased the amount of *R. solani* infection (disease score as described in Chapter 3), whilst it had no effect on Gatcher.

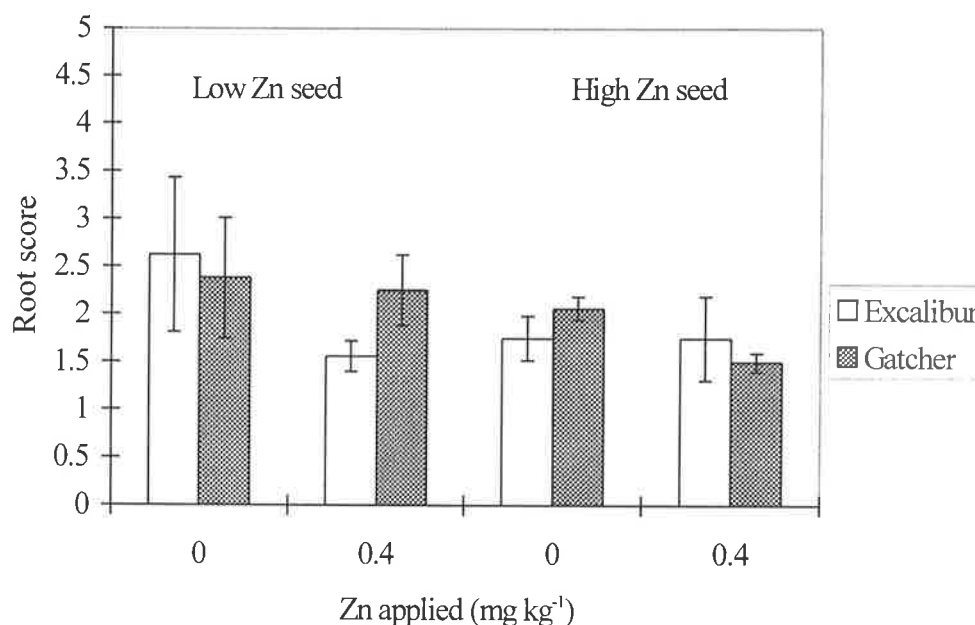


Figure 4.5. Disease severity of two cultivars of wheat seedlings (Excalibur and Gatcher) after 28 days of growth with 0 or 0.4 mg kg⁻¹ soil applied Zn and with 16 propagules of *R. solani* inoculum. Scoring system is as illustrated in Figure 1.4. Values are means of four replicates $\pm$ SE. Probabilities of treatment significance for disease score main effects were not significant at the $\alpha = 0.05$ level.

4.4 Discussion

The greatest yield of shoots from plants infected by *R. solani* was achieved when Zn was supplied via the soil rather than through the seed. These results suggest that plants developing from seed with a high seed Zn concentration will grow only marginally better than a plant developing from seed with a low seed Zn concentration when grown in Zn-deficient soil colonised by *R. solani*. When the soil is fertilised with Zn this marginal difference in growth is no longer evident. There is no apparent protective effect of Zn supply against *Rhizoctonia* infection as evidenced by no interactive effect in Figure 4.1.

Adequate nutrition does improve seedling vigour and growth. Zinc is readily mobilised into the embryo from other seed parts, and it has been demonstrated, in soybean, that as much as 70% of Zn stored in the seed is water-extractable (Khan and Weaver, 1989). It is therefore likely that while seed Zn is critical for early seedling growth, after a period of time this source of Zn is exhausted. Soluble carbohydrates are typically the first reserves to be depleted following seed imbibition (Bewley and Black, 1978, 1985) and the

rate of depletion is dependent on seed size, temperature and environmental conditions. Recently Moussavi-Nik *et al.* (1997, 1998) showed that almost the entire endosperm Zn reserves were depleted within 10 days from imbibition whilst little was mobilised from the largest reserve, the seed coat. Symptoms of Zn deficiency in the present study were evident 11-13 days after sowing and it is proposed that the seed Zn source had been exhausted by this time. Thereafter the seedling was reliant on the soil environment for a supply of Zn.

Using the definition of Zn efficiency by Graham *et al.* (1992), Excalibur is more Zn-efficient than Gatcher (on untreated soils) when the performance of the two cultivars is compared from calculations of shoot yield in a Zn-deficient soil (Figure 4.1). The effect of low soil Zn status on vegetative yield was more dramatic in Gatcher, which has a Zn efficiency of 58%, compared with Excalibur, which has a Zn efficiency of 73% in the present study. However, Excalibur and Gatcher demonstrated a similar Zn efficiency (96% and 92% respectively) in terms of root dry matter production. This contradicts previous data that has demonstrated Excalibur to produce a larger root system than Gatcher when grown to a similar age in Zn-deficient conditions (Rengel and Graham, 1995). The greater Zn efficiency displayed by Excalibur over Gatcher, in this experiment, is suggested to be partly due to differences in root morphology of the two cultivars. The increased Zn efficiency of Excalibur over Gatcher was proposed to arise from an ability to produce a root system with a higher proportion of fine roots, giving a higher surface area to volume ratio (Dong *et al.*, 1995; Holloway, 1996). If this is the case then Excalibur having a greater proportion of finer roots than Gatcher, will be better able to 'escape' infection by producing lots of surface area with little investment of resources.

Previous research has demonstrated Excalibur to be a most consistently, highly Zn-efficient cultivar over two years and at two different field sites (Graham *et al.*, 1992). In the same experiments, Gatcher was shown to be consistently Zn-inefficient. Data from the present experiment reflects these observations but the differences in efficiency between the two cultivars were not as large as expected.

Zinc-deficient plants of both cultivars had an increased root/shoot ratio (Figure 4.3), which became even more apparent when plants were infected by *R. solani*. There

were no cultivar differences in the root/shoot ratio of plants growing in Zn-limited conditions. The increased root/shoot ratio during Zn deficiency may represent a greater root mass to allow the plant to explore a greater area of soil for the extraction of Zn and, in the case of *R. solani* infection, give the plant a better chance of escaping infection or surviving the infection. Crop-pathogen interrelations often result in an increase in root/shoot ratio (Rabbinge and Tijdsdijk, 1981). However the various processes that play a role in this observed increase are not understood. The shoot dry matter of Gatcher showed a greater decrease in yield than Excalibur when infected by *R. solani* at high Zn levels. This suggests that Gatcher is more sensitive than Excalibur to *R. solani* infection.

The Zn concentration of both cultivars does not fall below critical in either cultivar in the presence of *R. solani* inoculum, therefore Zn deficiency has not been promoted in the present experiment, hence fungal-induced Zn deficiency is not the cause of the observed yield reductions. Plants infected with *R. solani* have reduced whole shoot Zn concentrations (Figure 4.4), however this reduction is not severe enough to cause the yield decline observed in Figure 4.1 (Reuter and Robinson, 1997).

Agronomically, cultivars that are more tolerant of infection by *R. solani* may be of major importance for plant breeding. The current experiments tend to implicate a level of tolerance of wheat of *R. solani* infection, which is reliant on Zn nutrition. Therefore, further field experimentation would establish if reduction in grain yield due to *R. solani* is in fact less in Zn-sufficient plants compared to Zn-deficient plants. Cultivar differences between tolerance levels to take-all (*Gaeumannomyces graminis* var *tritici*) have been demonstrated (Pedler, 1994). The screening of a number of Australian wheat cultivars showed that Mn-efficient wheats are significantly less susceptible to infection by take-all (Pedler, 1994). Similarly, a cultivar that is tolerant of infection by *R. solani* could be so due to a superior mechanism of Zn uptake from a deficient soil and/or owing to a larger root system. Studies to determine if the increase in yield observed in wheat plants grown with applied Zn is a function of Zn uptake or due to an increased root system in Zn-adequate plants allowing greater uptake of nutrients in general, will be done. Also, studies

will be carried out to determine if another nutrient (phosphorus) when applied to infected plants has the same effect on yield.

Chapter 5

The effect of Zn and P supply and *R. solani* infection on root system morphology of wheat

5.1 Introduction

Root architecture and morphology are important elements of whole plant nutrient uptake (Nielsen *et al.*, 1994). There is evidence to suggest that plants, supplied with adequate nutrients, are capable of outgrowing disease (see Graham, 1983). The ability of a plant to 'escape' disease by producing new roots to replace those destroyed by disease is increased with adequate nutrient supply (Huber, 1981). It has been shown that root hairs and fine roots contribute a significant proportion of the nutrient uptake ability of plants (Nable and Webb, 1993), and they are most important for diffusion-limited nutrients, such as Zn and P (Osborne *et al.*, 1993). Greater fine-root proliferation increases the extent of soil exploration (Wullschleger *et al.*, 1994) and may assist in overcoming nutrient limitations that would otherwise constrain plant growth. It was hypothesised at the end of Chapter 4 that differences in tolerance to *R. solani* between cultivars in nutrient-deficient soils could be due to differences in root physiology and morphology. Phosphorus is known to have dramatic effects on root systems (Föhse and Jungk, 1983; Bates and Lynch, 1996); therefore the effect of added P is to be investigated in this chapter.

Rhizoctonia solani prunes the root system of cereal plants by inducing cortical rotting. However, data obtained in Chapters 3 and 4 demonstrated that reductions in shoot dry matter production are not a consequence of nutrient deficiency owing to a pruned root system because shoot Zn concentrations were adequate. It is hypothesised that a cereal plant, infected with *R. solani*, compensates for the loss of nutrient uptake by increasing root branching and by increasing the capacity of the remaining roots to take up nutrients.

An adequately nourished plant is thought to produce more roots of which a greater number will escape infection (Garrett, 1950). Thus, a well-nourished plant will still

produce a significant yield in spite of losing a portion of its root system (Garrett, 1970). This is an example of disease escape where a well-fertilised plant may possess a greater number, but not necessarily a greater proportion, of functional roots than a deficient plant (Garrett, 1950). Successful acquisition of water and nutrients by plants requires that functional roots be present within the soil profile and distributed in a manner to facilitate the gathering of limited soil resources (Wulfschleger *et al.*, 1994).

The present experiments were conducted to determine if Zn is unique in improving yield of wheat infected by *R. solani*, or if the addition of P can also improve yield in *R. solani* infected plants when plants are grown at a limiting P supply. The relative mobility of phosphorus in soils is low, thus making its acquisition highly dependent on the spatial and temporal pattern of soil exploration by the roots system.

It is hypothesised that P deficiency reduces plant tolerance to *R. solani* as does Zn deficiency. The present experiments investigate the influence of *R. solani* infection and nutrient deficiency on root morphology.

Experiment 1. Zn and root morphology

5.2 Aim

The aim of the following experiment was to test the hypothesis that the severity of disease, caused by *R. solani*, is not correlated with the Zn concentration in plant tops but instead may be related to root architecture.

5.3 Materials and Methods

Experimental design and treatment

A factorial experiment was conducted with four levels of added Zn (0, 0.05, 0.2 and 2 mg Zn kg⁻¹ air-dry soil), two inoculum densities (0 and 16 propagules (millet seed) kg⁻¹ soil), and one wheat cultivar (Songlen). A total of eight treatments were tested with five replicates. The 2 mg Zn kg⁻¹ represents a non-limiting supply of Zn and the intention

was to increase the range of applied Zn. Songlen was chosen as the cultivar so that direct comparisons with the work of Thongbai *et al.* (1993b) could be drawn.

Seed

Wheat seeds (*Triticum aestivum* L. cv Songlen) were surface-sterilised and germinated as described previously (Chapter 3).

Procedure

One kg of dry Laffer sand (1.12 kg wet soil at field capacity) was placed in pots as described in Chapter 3. Basal nutrient solutions, pot preparation, inoculum and inoculum colonisation for both experiments was identical to the procedure described in Chapter 4. Zinc treatments were obtained by applying $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0, 0.05, 0.2 and 2 mg kg^{-1} Zn). Five pre-germinated seeds were sown in each pot and water added to 12 % moisture (w/w) content (field capacity). After 7 days, the pots were thinned to four plants per pot. Plants were harvested 28 days after sowing, and the nutrient status of shoots was determined using ICP analysis of dry material.

Data collection

At harvest, shoots were removed, dried at 65 °C for 48 hours and weighed. Soil was washed off the roots with RO water and the plants had their average seminal root length measured. The numbers of spear tips on the root systems were also counted. Roots were sub-sampled for determination of total root length and total infected root length by the line intersection method of Tennant (1975).

Experiment 2. P and root morphology

5.4 Aim

The aim of Experiment 2 was to investigate if P deficiency reduces plant tolerance to *R. solani* as does Zn deficiency.

5.5 Materials and Methods

Experimental design and treatment

Two P treatments were imposed: 8.5 and 34 mg P kg⁻¹ air-dry soil, considered to be deficient and sufficient for growth (J. N. Pearson, pers. commun.), respectively, with two inoculum densities (0 and 16 propagules (millet seed) kg⁻¹ soil), and one wheat cultivar (Songlen). A total of 4 treatments were created and replicated four times.

Surface sterilisation of Seed

As in experiment 1.

Procedure

Phosphorus treatments were obtained by applying KH₂PO₄ to Laffer sand. Basal nutrients were as described previously, and Zn was applied at a rate of 0.6 mg Zn kg⁻¹. Five pre-germinated seeds were sown into individual pots. After sowing, pots were watered to 12 % moisture content (w/w) and grown in a glasshouse (May to June). After 7 days, seedlings were thinned to two per pot and were harvested 28 days after sowing.

Data collection

Data collection was as in experiment 1; in addition, the number of 3rd-order laterals was counted on the two most developed seminal roots. The average length of 1st-order laterals was determined for the 40 longest laterals on the two most developed seminal roots. A further sub-sample of roots was then dried as described above and weighed.

5.6 Results: Experiment 1

Plant growth

Shoot dry weight of Songlen was significantly increased ($P = 0.0001$) with an increase in the Zn application rate, irrespective of the presence of *R. solani* inoculum (Figure 5.1). Root dry weight was not significantly affected ($P = 0.28$) by increasing soil Zn concentration in either control or *R. solani* infected plants (Figure 5.1).

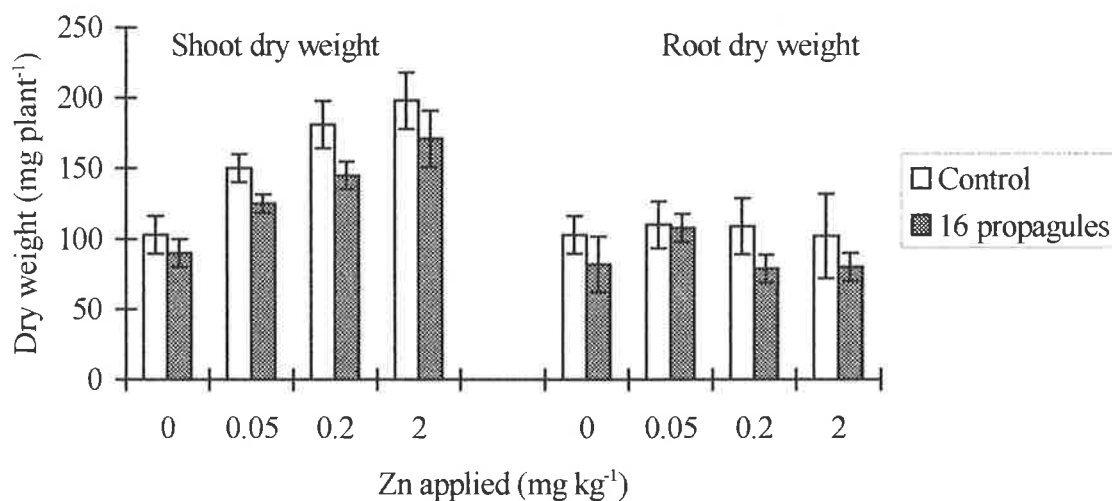


Figure 5.1. Shoot and root dry weight of Songlen wheat grown for 28 days with 0, 0.05, 0.2 or 2 mg Zn kg⁻¹ and with 0 or 16 propagules of *R. solani* inoculum. Values are means of five replicates $\pm$ SE.

Probabilities of treatments significantly influencing **shoot dry weight**: Zn treatment, $P = 0.0001$; Rhizoctonia, $P = 0.002$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

Probabilities of treatments significantly influencing **root dry weight**: Zn treatment, $P = 0.28$; Rhizoctonia, $P = 0.05$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

Root morphology

A trend of increasing root length with Zn application to the soil was observed although this was not statistically significant (Table 5.2). Zinc applications to wheat plants infected by *R. solani* significantly increased the total root length. *Rhizoctonia solani* infection decreased root length of Songlen wheat when no Zn was added but increased root length with Zn application. Seminal root length of control plants was significantly increased at the highest rate of soil-applied Zn in comparison to no applied Zn. *Rhizoctonia solani* infection significantly decreased mean seminal root length at all Zn rates (Table 5.2). No difference in the number of laterals per cm of seminal root was observed with an increase in soil-applied Zn. An increase in the number of laterals per unit length of seminal root was evident in plants infected with *R. solani* at higher rates of soil-applied Zn. An index of *R. solani* infection (number of spear tips) and the total root length of infected roots remained similar with an adequate supply of Zn (Table 5.2).

Table 5.2. The effect of Zn supply on **root length** (cm) of wheat (cv. Songlen) grown with control (0 propagules) and 16 propagules of *R. solani* inoculum 28 days after sowing. Values are means of five replicates $\pm$ SE.

<i>R. solani</i> inoculation	Zn applied (mg kg ⁻¹)							
	0		0.05		0.2		2	
	Control	<i>R. solani</i>	Control	<i>R. solani</i>	Control	<i>R. solani</i>	Control	<i>R. solani</i>
Root length (1)	510 $\pm$ 49	375 $\pm$ 67	538 $\pm$ 27	596 $\pm$ 55	564 $\pm$ 52	592 $\pm$ 22	575 $\pm$ 14	852 $\pm$ 128
Mean seminal root length (2)	15 $\pm$ 1	10 $\pm$ 1.3	16 $\pm$ 1.1	11 $\pm$ 1.7	16 $\pm$ 1	9 $\pm$ 1.3	23 $\pm$ 1.4	8 $\pm$ 2.6
Number of laterals per cm of seminal root (3)	3	5.3	3.4	4.5	3.4	7.2	2.2	7.6
Number of spear tips/root system (4)	0	11.1 $\pm$ 1.6	0	10.1 $\pm$ 1.2	0	10.4 $\pm$ 1.3	0	12.1 $\pm$ 1.2
Infected root length/root system	0	25 $\pm$ 3	0	29 $\pm$ 3	0	29 $\pm$ 2	0	29 $\pm$ 2

(1) Probabilities of treatments significantly influencing Root length: zinc treatment* Rhizoctonia, $P = 0.0097$.

(2) Probabilities of treatments significantly influencing Mean seminal root length: zinc treatment* Rhizoctonia, $P = 0.0150$.

(3) Probabilities of treatments significantly influencing Number of laterals: zinc treatment* Rhizoctonia, $P = 0.0181$.

(4) Probabilities of treatments significantly influencing Number of spear tips: zinc treatment* Rhizoctonia, $P = 0.0001$.

Table 5.3. The effect of Zn supply and *R. solani* infection on **Zn concentration** in whole shoots of Songlen wheat 28 days after sowing. LSD_{0.05} is for the interaction Zn applied × *R. solani*. Values are means of five replicates.

		Zn applied (mg kg ⁻¹)								LSD 0.05
		0		0.05		0.2		2		
		Control	<i>R. solani</i>	Control	<i>R. solani</i>	Control	<i>R. solani</i>	Control	<i>R. solani</i>	
<i>R. solani</i> inoculation										
Zn concentration (mg kg ⁻¹)		9.0	8.8	15.3	15.1	21.7	24.3	91.5	92.0	3.1

Nutrient status

Soil-applied Zn significantly increased shoot Zn concentration of Songlen in both control and *R. solani* infected plants ($P = 0.0001$) (Table 5.3). *Rhizoctonia solani* infection did not affect shoot Zn concentration of Songlen ($P = 0.46$) (Table 5.3). No interaction between Zn application and *Rhizoctonia* infection was evident in relation to shoot Zn concentration ($P = 0.59$).

5.7 Results: Experiment 2

Plant growth

Shoot dry weight of Songlen wheat was significantly increased with an increase in P application, irrespective of the presence of *R. solani* inoculum (Figure 5.2). Shoot dry weight was not reduced by *R. solani* when P was supplied at an adequate level for growth. Shoot dry weight of Songlen was reduced by *R. solani* when P supply was marginal for growth (Figure 5.2), but this result is not statistically significant. The interaction between P and *R. solani* infection did not significantly affect shoot yields.

Root dry weight of Songlen was not significantly affected by increasing soil P concentration in either control or *R. solani* infected plants (Figure 5.2). *Rhizoctonia solani* infection reduced root dry weight of Songlen grown with a marginal P supply but the effect

was not significant. There was no reduction in root dry weight when plants grown in P-adequate soil were infected with *R. solani* (Figure 5.2).

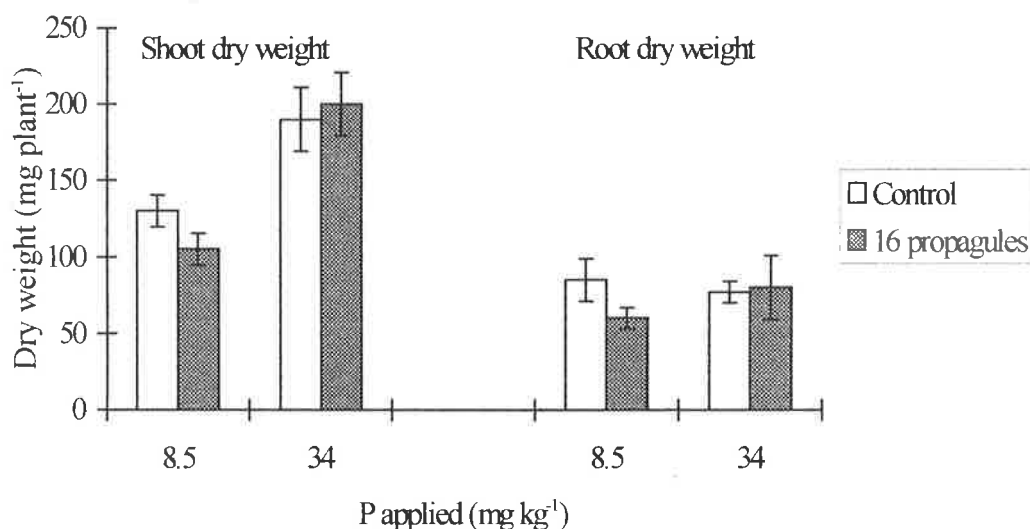


Figure 5.2. Shoot and root dry weight of Songlen wheat grown for 28 days with 8.5 or 34 mg P kg⁻¹ soil and either inoculated with control or 16 propagules of *R. solani*. Values are means $\pm$ SE of four replicates.

Probabilities of treatments significantly influencing **shoot dry weight**: P treatment, $P = 0.0004$; Rhizoctonia, $P = 0.6607$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

Probabilities of treatments significantly influencing **root dry weight**: P treatment, $P = 0.4512$; Rhizoctonia, $P = 0.2387$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

Root morphology

Adequate P application to the soil did not significantly influence the total root length of the control plants (Table 5.4). An increase in P supply to plants infected by *R. solani* significantly decreased total root length. *Rhizoctonia solani* infection slightly increased root length of Songlen grown at high P. Seminal root length remained unchanged by P application. Seminal roots of plants infected with *R. solani* were shorter than controls (Table 5.4). Phosphorus application decreased the mean lateral root length of control plants. *Rhizoctonia solani* infection increased mean lateral root length at low P but not at high P. No 3rd-order laterals were produced in control plants regardless of rate of P application. However, 3rd-order laterals were produced in *R. solani* infected plants

although there was no significant difference with P application (Table 5.4). *Rhizoctonia solani* infection was more severe when higher amounts of P were applied.

Table 5.4. The effect of P supply on root length (cm plant⁻¹) of wheat (cv. Songlen) grown with or without *R. solani* inoculum after 28 days. Values are means $\pm$ SE of four replicates.

<i>R. solani</i> inoculation	Phosphorus level (mg kg ⁻¹)			
	8.5		34	
	Control	16 propagules	Control	16 propagules
Root length (1)	1039 $\pm$ 139	1268 $\pm$ 134	854 $\pm$ 69	981 $\pm$ 46
Mean seminal root length (2)	27 $\pm$ 2	16 $\pm$ 1	31 $\pm$ 3	19 $\pm$ 2
Mean lateral root length (3)	71 $\pm$ 3	82 $\pm$ 4	57 $\pm$ 4	58 $\pm$ 1
Number of 3rd-order laterals (4)	0	8.0 $\pm$ 6	0	12.0 $\pm$ 4
Number of spear tips/root system (5)	0	9.9 $\pm$ 2	0	19.1 $\pm$ 1
Infected root length/root system (6)	0	4.1 $\pm$ 1	0	13.4 $\pm$ 2

- (1) Probabilities of treatments significantly influencing Root length: P treatment* *Rhizoctonia*, P = 0.6342.
- (2) Probabilities of treatments significantly influencing Mean seminal root length: P treatment* *Rhizoctonia*, P = 0.7162.
- (3) Probabilities of treatments significantly influencing Mean lateral root length: P treatment* *Rhizoctonia*, P = 0.1156.
- (4) (4) Probabilities of treatments significantly influencing Number of 3rd-order laterals: P treatment* *Rhizoctonia*, P = 0.0025.
- (5) Probabilities of treatments significantly influencing Number of spear tips/root system: P treatment* *Rhizoctonia*, P = 0.0002.
- (6) (6) Probabilities of treatments significantly influencing Infected root length/root system: P treatment* *Rhizoctonia*, P = 0.0002.

Nutrient status

Soil-applied P increased shoot P concentration of Songlen in both control and *R. solani*-infected plants (Table 5.5). *Rhizoctonia solani* infection reduced P concentration in shoots of plants grown at high P but had no effect at low P (Table 5.5).

Table 5.5. The effect of P supply and *R. solani* infection on **P concentration** in whole shoots of Songlen wheat 28 days after sowing. The $\text{LSD}_{0.05}$ value refers to significance of the interaction.

P applied (mg kg ⁻¹)					
		8.5		34	
<i>R. solani</i> inoculation	Control	<i>R. solani</i>	Control	<i>R. solani</i>	LSD _{0.05}
P concentration (g kg ⁻¹)	3.4	3.7	11.9	9.5	1.7

5.8 Discussion

Not surprisingly, the application of Zn and P to deficient soil increased shoot growth and the concentration of the respective nutrient in wheat shoots. This has been observed frequently in many species (Grewal *et al.*, 1996; Graham and Rengel, 1993; Föhse *et al.*, 1988). In the concentration range investigated in this study, it was observed that there was no effect of Zn and P fertilisation on root dry weight within four weeks of growth. Previous research on wheat has demonstrated an increase in root dry matter production with increased Zn application in plants aged between 3-6 weeks (Cakmak and Marschner, 1988c; Dong *et al.* 1995; Grewal *et al.* 1996). In comparison, prolonged phosphorus starvation of maize increased root dry weight and root length (Anghinoni and Barber, 1980). In 25-day-old *Arabidopsis thaliana*, an inadequate supply of P also resulted in an increase in root dry weight (Bates and Lynch, 1996). Plant species and cultivars within species respond to P deficiency by increasing the root/shoot ratio; the extent to which the ratio is increased depends on the severity of P deficiency. Whiteaker *et al.* (1976) found contrasting results with two cultivars of bean receiving adequate and deficient P supplies. Root dry weight of one cultivar (cv. 6) of bean was greatly reduced with a low P supply, whilst the root dry weight of another cultivar (cv. 11) nearly doubled.

The authors concluded that the capacity to distribute a higher proportion of photosynthates to the roots is obviously under genetic control and is an important aspect of phosphorus efficiency for plants grown in deficient soil. The efficiency of P acquisition and internal utilisation is dependent upon the level of nutrients supplied, plant age and also cultivar differences in nutrient efficiency (Marschner, 1995).

Although Zn and P application had no effect on root dry matter production in experiments described here, there was an effect on total root morphology. It is demonstrated that total root length increased with an adequate Zn supply (Table 5.2) or a lower P supply (Table 5.4). This contradicts other evidence that Zn deficiency increases root growth and decreases shoot growth (Rengel and Graham, 1995). However, this response is often seen in solution-cultured plants in Zn-deficient conditions (Cumbus, 1985; Robson and Snowball, 1990; Rengel and Graham, 1995) but not in soil-grown plants (Dong *et al.*, 1995), the later result being in accordance with the present study. This suggests that the partitioning of assimilates between roots and shoots under Zn deficiency can be quite different when plants are grown in soil compared to those grown in nutrient solution (cf. Rengel and Graham, 1995).

Low availability of P stimulates root hair elongation in many plants, which may have an adaptive significance in soil P acquisition (Föhse and Jungk, 1983). In addition, plants suffering from P deficiencies have been observed to produce longer, thinner roots compared with P-sufficient plants (Bates and Lynch, 1996). Longer, thinner roots may benefit the plant by increasing the effective surface area per gram of roots to take up nutrients, an especially important characteristic for the uptake of diffusion-limited nutrients (Dong *et al.* 1995). An increased fine root mass, however, does not necessarily imply a larger volume of soil occupied or exploited by the root system. A more direct assessment is provided by measures of root length and branching patterns (Norby, 1994). The longer root system of P-deficient plants in the present study is partly due to an increase in the mean length of 1st-order laterals and not due to an increase in seminal root length (Table 5.4). Increases in production of lateral roots under P deficiency have also been demonstrated in *Arabidopsis thaliana* (Bates and Lynch, 1996).

Rhizoctonia solani infection has been observed to decrease yields in cereals (Samuel and Garret, 1932; Murray, 1981; MacNish and Neate, 1996). In this study, *R. solani* infection lowered yields in those plants that were supplied with amounts of Zn that were bordering either side of critical values (0.05 and 0.2 mg kg⁻¹ Zn) and slightly decreased the yield of plants grown with marginal P. Nutrient concentrations of whole shoots indicated that the decrease in yield was not due to *R. solani* infection causing a nutrient deficiency. Zinc concentrations of whole shoots remained unchanged with *R. solani* infection over all Zn treatments (Table 5.5). Phosphorus concentrations in the shoot tissue of diseased plants did not fall below the critical ranges reported for wheat (Reuter and Robinson, 1997). An explanation of why plants did not display *R. solani*-induced deficiencies may be due to increased uptake by the newly formed roots so that the uptake of P or Zn per unit length of total root could be much higher in the infected plants compared to the controls. Yield losses, however were still observed. It is suggested that the losses occurred because of the initial reduction of nutrient supply from the pruned roots, but newly formed roots increased nutrient uptake and therefore restored the concentration of Zn or P within the shoots of infected plants. The differential partitioning of assimilates in plants infected by *R. solani* may also vary. Wall *et al.* (1994) investigated the effect of *R. solani* on field-grown wheat and observed reductions in yield of diseased plants. The diseased plants also had purple stems, usually associated with P deficiency. Wall *et al.* (1994) concluded that the *R. solani* infection induced P deficiency in wheat. The presented research demonstrates that *R. solani* infection decreases P concentration only under conditions of an adequate supply of P. However there was no significant decrease in yield suggesting that damage caused by *R. solani* did not cause P deficiency. However, the field experiments conducted (Wall *et al.*, 1994) may not be directly comparable to the pot experiments presented here due to possible differences caused by environmental factors.

Rhizoctonia solani infection did not reduce the yield of plants grown without applied Zn (see also Chapter 3). In this case, severe Zn deficiency may have limited growth to a greater extent than *R. solani*. Previous research by Thongbai *et al.* (1993b)

showed reductions in shoot dry weight of plants grown without applied Zn and infected by *R. solani*. However, during that research Songlen was grown for a period of 50 days compared to plants in this experiment that were grown for 28 days. The increase in the growth period of plants by Thongbai *et al.* (1993b) may have allowed *R. solani* to increase its colonisation of the plant and further damage the already weakened root system. Plants were not grown for 50 days in the current experiments as a comparison was required with previous chapters.

Adequately nourished (Zn or P) Songlen wheat responded to *R. solani* infection by altering root morphology, such as increasing lateral root production. The killing of root tips and other parts of roots by *R. solani* stimulate the formation of lateral roots, which may in turn be attacked by the fungus. The result is a rather characteristic, much-branched root system in diseased plants (Samuel and Garret, 1932). The increased production of laterals of infected Songlen plants is evident in Tables 5.2 and 5.3.

Plants responded similarly to the stress imposed by either P or Zn deficiency or *R. solani* infection. *Rhizoctonia solani* inoculation of Songlen wheat resulted in increased root length, in both the Zn and P experiments, an increase in the number of laterals per unit length of seminal root (Experiment 1) and an increase in the mean length of 1st-order laterals (Experiment 2). Infection by *R. solani* appears to have had the similar effects as P deficiency in that the plant grew more roots which were able to explore the soil. It is suggested that the production of more roots decreases the impact of infection, supporting the disease escape theory of Huber (1981).

These results suggest the plant is tolerating infection by *R. solani*. Tolerance is defined in this case as the ability of a plant to produce a significant yield when disease incidence is high. It was observed that there was more infection at higher Zn and P levels (Tables 5.2 and 5.3), indicating that Songlen wheat has no resistance mechanism to *R. solani* infection that is linked to an improvement in nutrition. The reduced infection of Zn- and P-deficient plants is not explained by a direct role of Zn in the saprophytic growth of the fungus as demonstrated by Thongbai *et al.* (1993c), or by a direct role of P in the saprophytic growth of the fungus (see experimental results in Appendix 1).

Possible reasons for decreased infection (infected root length (Table 5.3)) of P-deficient plants lie in the allocation of carbohydrates. In phosphorus-deficient plants, roots become the dominant sink not only for photosynthates but also for phosphorus from the shoot (Marschner, 1995). In this way carbohydrates are allocated to roots and as a result root length is increased with a corresponding decrease in shoot dry weight. It has become apparent in recent years that the carbohydrates in the cell wall function not only to maintain cell shape and integrity, but also may serve as signals in plants. Evidence that biologically-active oligosaccharides released from plant cell walls can serve as signals to regulate plant defence and plant growth and development has been found (Mohen and Hahn, 1993). Therefore carbohydrates may only increase disease tolerance by increasing root length but they may also regulate plant defence against disease.

Observation of results in Table 5.2 show that Zn-deficient plants (0 mg applied Zn) do not increase root length when infected by *R. solani*. It is known that Zn deficiency results in impaired photosynthesis in all plant species (Brown *et al.*, 1993). The proportion of carbohydrate allocation to roots may be decreased as root growth is proportional to photosynthesis (Marschner, 1995). Plants supplied with Zn have a greater root length and there is a further increase when plants are infected by *R. solani*. This data suggests that Zn deficiency results in a reduced allocation of carbohydrates to roots and hence impaired root growth. The impact of *R. solani* in increasing root growth is possibly a response by the plant to increase nutrient uptake capability to offset the impact of *R. solani* on nutrient uptake efficiency.

The ability of Zn-adequate plants to tolerate infection of *R. solani* may be due to the role of Zn in photosynthesis. The effect of Zn deficiency on photosynthesis is not only via carbonic anhydrase (as outlined in Literature review Section 2.2.3.1) but is also associated with a reduction in the activity of ribulose 1,5-bisphosphate carboxylase (RuBPC), which catalyses the initial step of photosynthetic CO₂ fixation. Generally, carbonic anhydrase is present in excess of what may be required for photosynthesis (Randall and Bouma, 1973), resulting in a relatively poor relationship between carbonic anhydrase activity and the photosynthetic rate. However, carbonic anhydrase activity is

much lower in wheat when compared to a number of other species (Makino *et al.*, 1992). Hence, the level of carbonic anhydrase activity may be more important for maintaining the photosynthetic rate in wheat than in other species (Rengel, 1995). Rengel (1995) showed the effect of Zn deficiency on carbonic anhydrase activity was greater in Zn-inefficient Durati than in Zn-efficient Warigal, indicating a positive relationship between carbonic anhydrase activity and Zn efficiency. The ability to maintain relatively greater carbonic anhydrase activities in the Zn-efficient cultivars may prove beneficial in maintaining the photosynthetic rate at a greater level, hence maintaining a greater carbohydrate allocation to roots. This process may prove valuable when wheat is infected by *R. solani*.

Results within this chapter indicate that root system morphology is dramatically affected by *R. solani* infection and these effects may play an important role in the increased tolerance to *R. solani* in wheat. Root systems vary greatly in size, lateral extent, depth, rooting intensity, proportion of fine and coarse roots, and other characteristics that might affect the relationship of photosynthate utilisation to soil resource acquisition. Reducing losses in yield caused by *R. solani* may be achieved by offsetting any nutrient limitations or by promoting cultivars that respond to nutrient deficiency by increasing their root length or by selecting cultivars that have an inherently longer root system. In each case, the disease may be tolerated and losses in yield reduced.

The next experimental chapter focuses on whether root morphology plays a role in increased tolerance to *R. solani* in different cultivars.

Chapter 6

The effect of Zn supply on seedling susceptibility to *R. solani* infection in three cultivars of wheat

6.1 Introduction

Cultivar tolerance to a particular disease may arise due to high efficiency in nutrient uptake or utilisation (Huber and Graham, 1998). Nutrient efficiency itself may be influenced by root morphology. In Chapter 5, the ability of wheat to tolerate infection by *R. solani* has been associated with differences in root morphology. Considerable variation in root development of wheat grown under Zn deficiency has been reported (Cumbus, 1985) as well as morphological differences in the roots of cultivars of wheat differing in Zn efficiency (Dong *et al.*, 1995). In particular, lateral root development (number and length) of wheat has been shown to increase under Zn deficiency (Cumbus, 1985). It has also been reported that root hair production is stimulated under P deficiency (Bates and Lynch, 1996). Therefore, root architecture is an important variable to consider when determining relationships between root disease and soil resource acquisition.

In screening for resistance to *R. solani* no consistent genetic differences were found among cereal cultivars (Purss and Dube, 1984; McDonald and Rovira, 1985; Neate, 1989). However, root morphology varies among cultivars as well as species (Hackett, 1968; Itoh and Barber, 1983; Fitter, 1991; Schwarz *et al.*, 1991). In previous chapters, the cultivar Excalibur, which is known to be Zn-efficient, was more tolerant of *R. solani* than Gatcher (see Chapter 4), and plants with a greater exploitation of soil (higher root length) were also more tolerant to *R. solani* (see Chapter 5). Differential root development between cultivars may not only affect nutrient uptake, but may also play an important role in differential sensitivity to nutrient deficiency and therefore consequently affect differential tolerance to soil-borne plant pathogens. No report on the relationship between

root growth, nutrient fertilisation and differential disease tolerance of crop cultivars can be found in the literature.

Here, it is proposed that tolerance to *R. solani* is associated with wheat cultivars that possess either increased root hair production, greater total root length, or inducible lateral root development. These cultivars may tolerate *R. solani* by increasing root length and root surface area for nutrient uptake (i.e. increased root length or lateral production), as well as efficiently exploiting nutrients in the soil through increased root hair production. Three cultivars of wheat were chosen on the basis of previously ascertained differences in root morphology to test this hypothesis.

The aim of the present work is to study root morphology of wheat cultivars and relate these findings to tolerance of *R. solani*.

6.2 Materials and Methods

Experimental design and treatment

A factorial experiment was conducted with four levels of added Zn (0, 0.05, 0.2 and 2 mg Zn kg⁻¹) applied as ZnSO₄, two inoculum densities (0 and 16 propagules (millet seed) kg⁻¹ soil), and three wheat cultivars (Songlen, Janz and RAC 655) with five replicates.

Cultivars

Three cultivars of wheat were chosen for the present experiment on the basis of their root mass. RAC 655 was selected for a greater root mass, Songlen for a medium level of root mass and Janz for a low root mass when grown at low levels of applied Zn for 4 weeks (Moussavi-Nik *et al.*, 1997). Seeds of uniform size (sieved to 2.5 - 3 mm) and similar low Zn concentration (mg Zn kg⁻¹) (14 ± 0.3 , 10 ± 0.5 and 13 ± 0.5 for Janz, Songlen and RAC 655, respectively) were used in this study. Seeds were surface-sterilised as described in Chapter 3 and germinated on moistened filter paper for 36 hours at 20 °C prior to sowing.

Procedure

Pot preparation was identical to that described in Chapter 4, Section 4.2, except KH_2PO_4 was applied at a rate of 90 mg kg^{-1} (a level considered to be adequate for optimal plant growth). Five seeds were sown to a pot, and after seven days seedlings were thinned to three per pot and grown for 28 days.

Root samples were analysed in the same manner as in Chapter 5. However, in addition, the production of root hairs was compared between treatments. The root systems were placed into shallow trays in water and the roots separated to allow visual comparison of root hair production. Root systems were visually grouped into one of the following categories using a magnifying glass:

1. No root hairs or most root hairs less than 0.5 mm long;
2. Moderate amount of root hairs between 0.5 mm and 1 mm;
3. Many root hairs with a length usually exceeding 1 mm.

Shoot Zn concentrations were analysed as in previous chapters.

6.3 Results

Shoot growth

Shoot dry weight of all cultivars increased with an increase in the Zn application rate, irrespective of *R. solani* inoculation (Figure 6.1). In general, RAC 655 produced more dry matter than Janz and Songlen when grown in Zn-deficient soil with Janz producing more dry matter than Songlen at the two lowest rates of Zn. However, wheat plants grown with luxurious Zn all grew similarly (Figure 6.1).

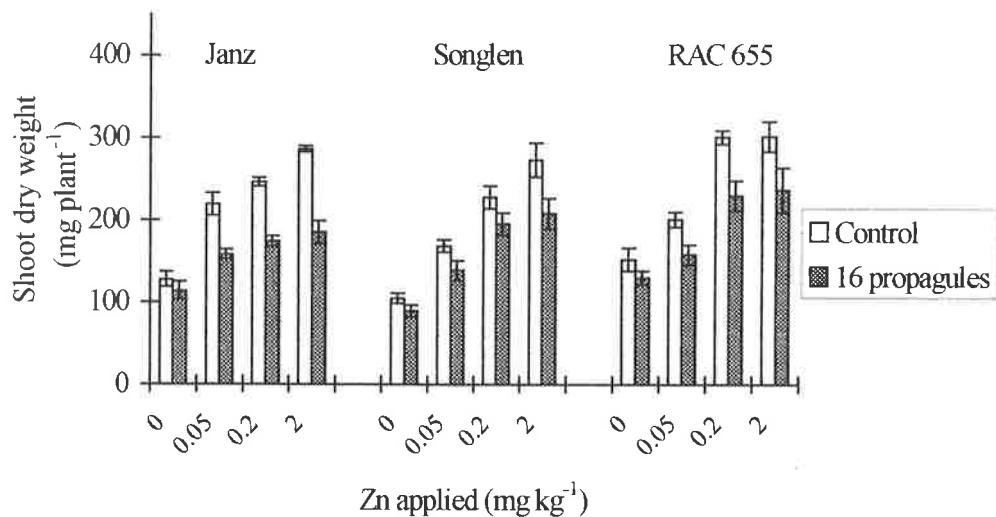


Figure 6.1. Shoot dry weight (mg plant⁻¹) of three wheat cultivars grown in soil fertilised with four Zn rates with or without *Rhizoctonia* inoculum. Values are means of five replicates $\pm$ SE. Probabilities of treatments significantly influencing shoot dry weight: cultivar, Zn treatment and *Rhizoctonia*, $P = 0.0001$; Cultivar $\times$ Zn, $P = 0.05$; zinc $\times$ *Rhizoctonia*, $P = 0.002$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

Shoot dry matter of all three cultivars decreased when infected with *Rhizoctonia* although differences were small at the lowest Zn rates. The magnitude of the decrease at low level of applied Zn (0.05 and 0.2 mg Zn kg⁻¹) was greater in Janz and RAC 655 than in Songlen (Figure 6.1). However, RAC 655 continued to produce the greatest shoot dry matter (Figure 6.1).

Root length

The total root length of all three cultivars was lowest when the plants received no additional Zn. Songlen produced the shortest roots under these conditions (Figure 6.2). All cultivars produced more roots when Zn was applied at the level of 0.05 mg kg⁻¹. Little increase in root length was observed with increasing Zn supply above that rate. Infection by *R. solani* either had little effect or increased root length of all cultivars over all Zn treatments.

RAC 655 displayed the shortest infected root length when grown with no added Zn, whilst Janz had the longest length of infected roots under these conditions (Table 6.1). Infected root length of Janz and RAC 655 was unaffected by Zn treatment. The length of infected roots in Songlen significantly decreased with a Zn application rate of 2 mg kg⁻¹ (Table 6.1).

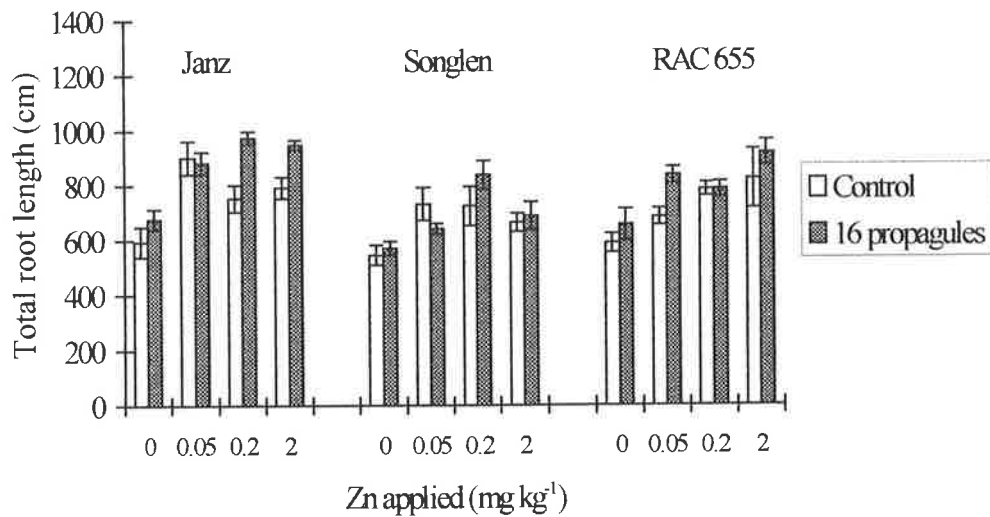


Figure 6.2. Total root length (cm) of wheat cultivars Janz, Songlen and RAC 655 grown in 4 levels of soil-applied Zn after 28 days growth with and without *R. solani*. Values are means of five replicates.

Probabilities of treatments significantly influencing total root length: cultivar and Zn treatment, $P = 0.0001$; Rhizoctonia, $P = 0.0006$. Cultivar $\times$ Zn, $P = 0.0503$. The other interactions were not significant at the $\alpha = 0.05$ level.

Table 6.1. Infected root length plant⁻¹ (cm) of Janz, Songlen and RAC 655 wheats grown at four levels of soil-applied Zn for 28 days. Values are means of five replicates.

	Zn-applied (mg kg ⁻¹)			
	0	0.05	0.2	2
Janz	64	66	64	77
Songlen	49	30	59	34
RAC 655	34	34	34	43
LSD _{0.05} Cultivar × Zn = 13.6				

Seminal root length of Janz and Songlen significantly increased only at the highest rate of Zn application (Table 6.2). RAC 655 initially increased seminal root length with the application of 0.05 mg kg⁻¹ Zn but then remained unaffected by a higher rate of applied Zn. *Rhizoctonia solani* infection significantly reduced seminal root length over all Zn treatments in all cultivars. Infection by *R. solani* reduced seminal root length of all plants in all Zn treatments to the same length regardless of the differences in lengths of seminal roots between cultivars in uninfected control plants.

Table 6.2. Seminal root length plant⁻¹ (cm) of Janz, RAC 655 and Songlen grown for 28 days at 0, 0.05, 0.2 or 2 mg Zn kg⁻¹ and with or without *R. solani* inoculum. Values are means ± SE of five replicates.

	Zn applied (mg kg ⁻¹)							
	0		0.05		0.2		2	
	Control	<i>R. solani</i>	Control	<i>R. solani</i>	Control	<i>R. solani</i>	Control	<i>R. solani</i>
Janz	16 ± 1.3	7 ± 0.7	16 ± 0.7	8 ± 1.2	16 ± 0.2	8 ± 0.2	20 ± 1.3	7 ± 0.4
Songlen	13 ± 0.9	7 ± 0.8	13 ± 0.9	12 ± 0.4	14 ± 0.6	8 ± 0.3	16 ± 0.8	9 ± 3.4
RAC 655	13 ± 0.7	7 ± 2.1	15 ± 0.3	8 ± 2.2	15 ± 0.5	9 ± 0.7	15 ± 0.3	8 ± 0.4

Root hair estimation

The three cultivars differed markedly in their production of root hairs under all conditions (Figure 6.3). Janz produced the most root hairs over all Zn treatments and was

not significantly affected by *R. solani*. Songlen produced a medium number of root hairs in the presence of *R. solani*. RAC 655 produced few root hairs in control plants; however, infection by *R. solani* caused the plants to produce more root hairs at all but the highest Zn level.

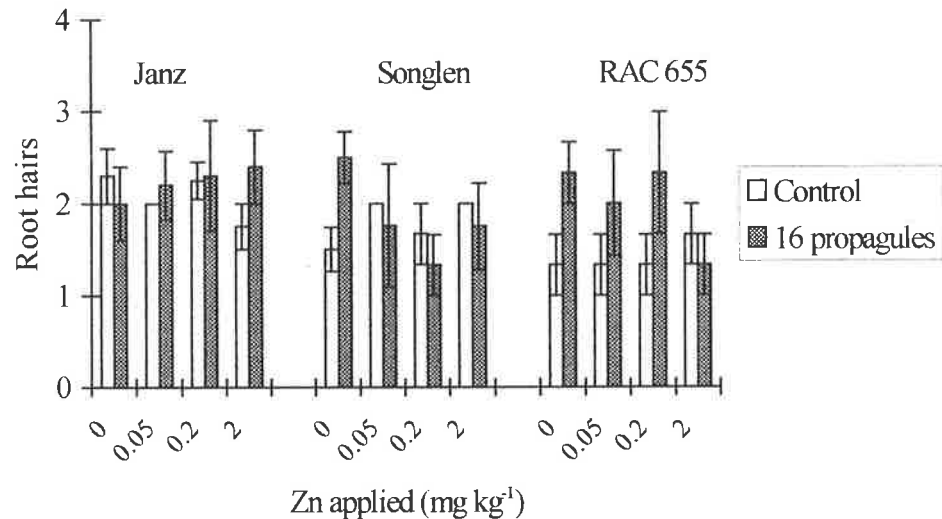


Figure 6.3. Root hair quantification of three cultivars of wheat grown for 28 days. Plant root systems were scored on a scale of 1-3. 1 = little or no root hairs, 2 = medium level of root hairs and 3 = many root hairs. Values are means of three replicates $\pm$ SE.

Zinc status

The application of Zn to Zn-deficient soil significantly increased Zn content and concentration in shoots of all cultivars (Figures 6.4 and 6.5). The zinc application rates of 0 and 0.05 mg kg⁻¹ were deficient for optimal shoot growth of wheat, the application rate of 0.2 mg Zn kg⁻¹ bordered on critical, and the application rate of 2 mg Zn kg⁻¹ exceeded that required for adequate growth. Songlen displayed a higher shoot Zn concentration than RAC 655 and Janz wheat when grown in soil with a adequate Zn supply but not at lower Zn rates. Shoot Zn concentrations and contents of Janz, RAC 655 and Songlen were significantly reduced by *R. solani* infection at the highest level of applied Zn (Figures 6.4 and 6.5). At that level of soil Zn, Songlen was able to extract more Zn from the soil and

maintain a higher shoot Zn concentration compared to Janz or RAC 655 in the presence of *R. solani* (Figure 6.5).

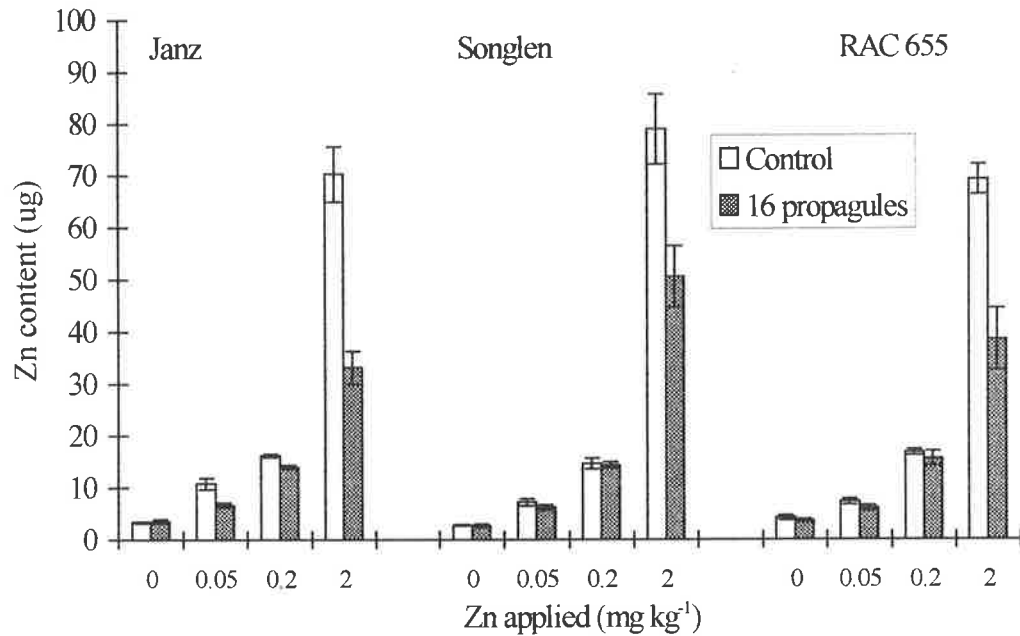


Figure 6.4. Zn content (μg) in shoots of three wheat cultivars, grown with 0, 0.05, 0.2 and 2 mg Zn kg^{-1} . Values are means of 5 replicates $\pm$ SE. Probabilities of treatments significantly influencing Zn content: cultivar, $P = 0.1556$; Zn treatment and Rhizoctonia, $P = 0.0001$. Cultivar $\times$ Zn, $P = 0.0009$ and zinc $\times$ Rhizoctonia, $P = 0.0001$. The other interactions were not significant at the $\alpha = 0.05$ level.

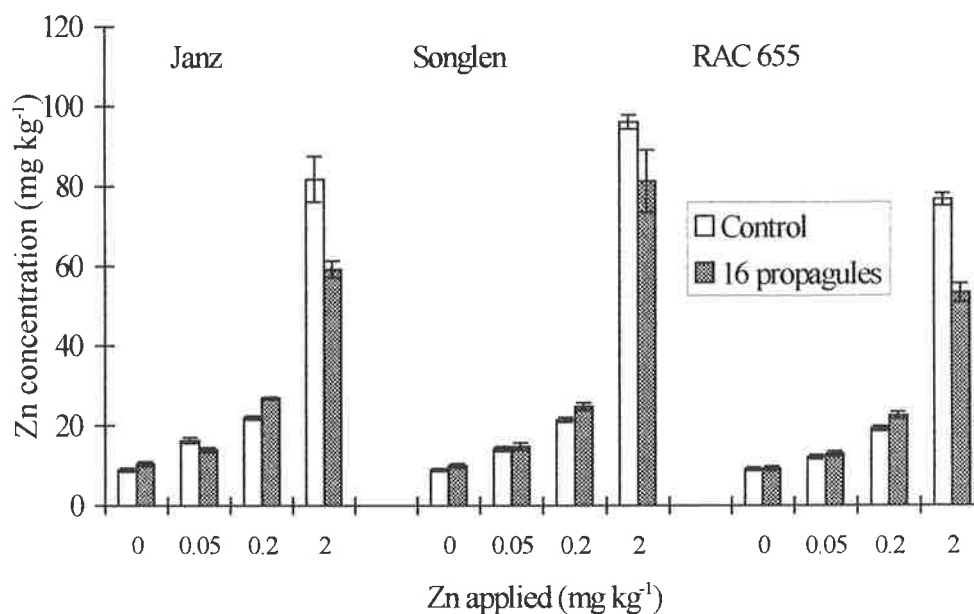


Figure 6.5. Zn concentration (mg kg^{-1}) in shoots of three wheat cultivars, grown with 0, 0.05, 0.2 and 2 mg Zn kg^{-1} . Values are means of 5 replicates $\pm$ SE. Probabilities of treatments significantly influencing Zn concentration: cultivar, Zn treatment and Rhizoctonia, $P = 0.0001$. cultivar $\times$ Zn and zinc $\times$ Rhizoctonia, $P = 0.0001$. The highest order interaction was not significant at the $\alpha = 0.05$ level.

The Zn influx ($\mu\text{g Zn}$ taken up per cm of root) of all cultivars grown at a low level of applied Zn was not significantly different; however, the Zn influx of Songlen was greater than Janz or RAC 655 at the highest level of applied Zn (Figure 6.6). Inoculation with *R. solani* had no effect on the Zn influx of plants grown at a low level of applied Zn but did reduce the Zn influx of roots at a high level of applied Zn (Figure 6.6). When infected with *R. solani*, Songlen maintained a much greater Zn influx than Janz or RAC 655 at high Zn.

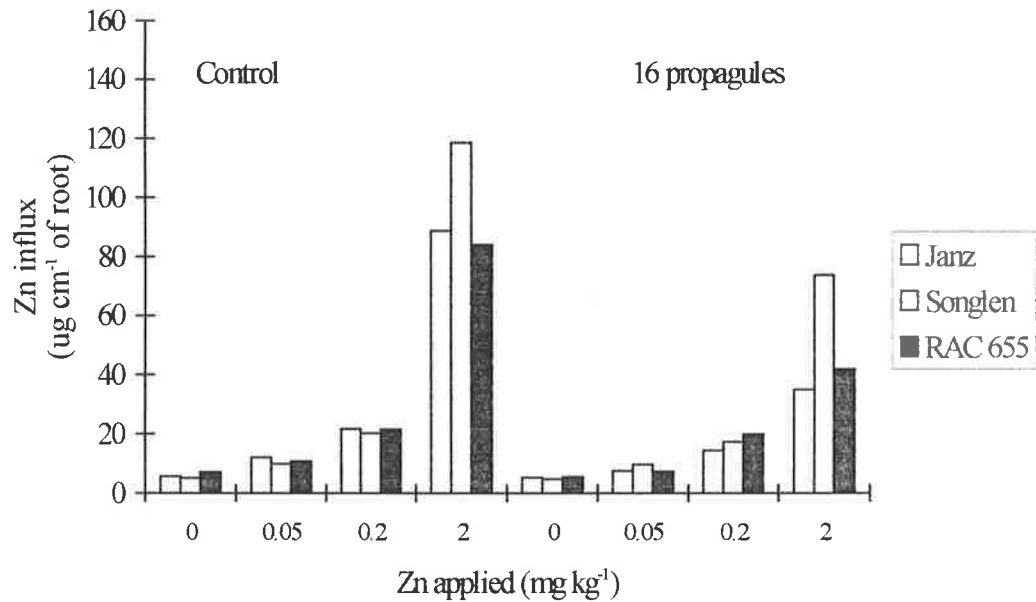


Figure 6.6. Zinc influx ($\mu\text{g cm}^{-1}$ of root) in three cultivars of wheat inoculated with and without *R. solani*.

Figure 6.7 displays the relationship between the percentage decrease in shoot growth due to *R. solani* inoculum treatment, and the percentage decrease in Zn influx due to *R. solani* inoculum treatment taken for all three cultivars tested. There was a direct correlation between the decrease in Zn influx and the decrease in shoot growth in the presence of *R. solani* inoculum.

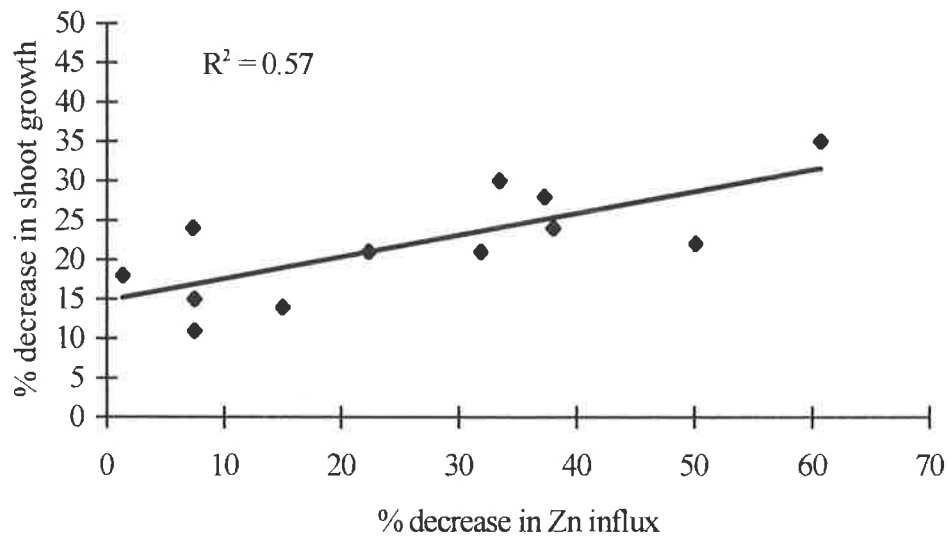


Figure 6.7. Percentage decrease in shoot growth versus percentage decrease in Zn influx of control plants relative to *R. solani* infected plants. The graph contains data for all three cultivars.

6.4 Discussion

Where nutrient deficiencies exist, the resulting symptoms on the plant, such as restricted growth and reduced yield may, if associated with the occurrence of root rot lesions in the plants, be wrongly attributed to the direct effects of root-rot organisms. Alleviation of the soil nutrient deficiency by a suitable treatment may therefore decrease the apparent severity of the disease without actually decreasing the number of lesions on the roots of the affected plants (Millikan, 1942).

Root morphology can influence nutrient uptake. When Zn influx per unit length of root is calculated, it is observed that Songlen has a greater Zn influx than Janz or RAC 655 when Zn is not limiting. *Rhizoctonia solani* inoculum is associated with a reduction in Zn influx and, furthermore, there is a direct relationship between the percentage decrease in Zn influx and the percentage decrease in shoot growth (Figure 6.7).

The term Zn efficiency needs to be clarified within the present study. Zn efficiency can be termed as a mechanism that allows a greater Zn uptake per unit area of root or a mechanism that allows a cultivar to produce higher yield when exposed to Zn-

deficient conditions. The ultimate assessment of Zn efficiency (Graham and Rengel, 1993) is in a field experiment where the soil is deficient enough in plant-available Zn to seriously limit the yield of some cultivars. The introduction of a +Zn treatment (adequate Zn) will define the yield potential and allow the calculation of the Zn efficiency index:

$$\text{Zn efficiency} = \frac{\text{Yield (-Zn)}}{\text{Yield (+Zn)}} * 100$$

Using this system of calculating Zn efficiency, Janz and RAC 655 have similar Zn efficiencies whilst Songlen displays the lowest Zn efficiency ranking. However, when Zn influx is assessed Songlen is able to extract a greater concentration of Zn from the soil but only at a high rate of applied Zn. The mechanism of Zn efficiency has been shown in this study to be disrupted by *R. solani*. *Rhizoctonia solani* tolerance is directly related to the ability of the diseased plant to maintain Zn uptake. In this particular study it is demonstrated that Songlen is more tolerant of *R. solani* than either Janz or RAC 655, which confers with previous experimental data by Thongbai *et al.* (1993b). However, Songlen is not considered to be a truly Zn efficient cultivar by definition (Graham and Rengel, 1993).

The following experimental chapter is aimed at shedding more light on the relationship between Zn influx and transport with tolerance to *R. solani* infection.

Chapter 7

Does Zn transport differ in cultivars of wheat when infected with *Rhizoctonia solani*?

7.1 Introduction

There is difficulty in interpreting static measures of root mass when root growth is episodic or when significant root death occurs (Norby, 1994), which is the case when studying root diseases. Yield loss caused by infection of *R. solani* was shown to be linearly related to decreased Zn uptake.

Plant cultivars of many crop species show important differences in their uptake, translocation, accumulation, and use of the essential mineral elements (Clark, 1983). The ability of a plant cultivar to carry out the above activities efficiently is hypothesised to increase their tolerance to root disease. The potential of a plant to absorb nutrients depends on the amount of absorbing root surface and the activity of those roots to facilitate absorption (Chapin, 1991). Therefore changes in root morphology are not solely correlated with increased tolerance to disease but are one factor of many influencing tolerance. It has been demonstrated (Chapter 6) that wheat cultivars differ in their response to *R. solani* (*R. solani* tolerance) but the differences are not correlated to changes in root morphology. Therefore it is proposed that tolerance to *R. solani* may occur in those cultivars which have an inherently greater ability to take up or transport Zn. It is hypothesised that these cultivars will show a smaller loss in yield when infected by a root disease than their less efficient counterparts.

The following experiment investigated whether Zn transport potential of a cultivar affects the tolerance to *R. solani* infection.

7.2 Materials and Methods

Experimental design

A factorial experiment combining three rates of soil-applied Zn (0, 0.04 and 0.8 mg kg⁻¹ Zn), two levels of *R. solani* inoculation (0 and 16 propagules) and three cultivars (Excalibur, Songlen and Gatcher) was conducted in pots under controlled conditions as described previously. Cultivars are not consistent with Chapter 6 as seed of similar Zn content was limiting. A total of 48 treatments were created and replicated four times.

Inoculum and growth conditions

All methods were the same as described previously (Section 3.2.3).

7.3 Results

Plant growth

Symptoms of Zn deficiency, in nil Zn treatments, were first apparent in shoots of Gatcher and Songlen 11 days after sowing, and then Excalibur 15 days after sowing. Symptoms of Zn deficiency were severe by day 14 in Gatcher and Songlen (in nil Zn treatments). Excalibur, grown in soil with no applied Zn, did not show severe symptoms until day 22. Shoot dry weight of plants at 0 mg Zn kg⁻¹ indicates that the soil was deficient for growth.

Soil-applied Zn increased shoot dry weights in all cultivars, irrespective of *R. solani* inoculation (Figure 7.1). However, a significant interaction of Zn and *R. solani* inoculum is apparent. Excalibur obtained the greatest yield of all cultivars when grown in Zn-adequate soil.

Shoot dry matter of Songlen and Gatcher was reduced when infected with *R. solani*, but not to the extent of the reduction observed in Excalibur (Figure 7.1).

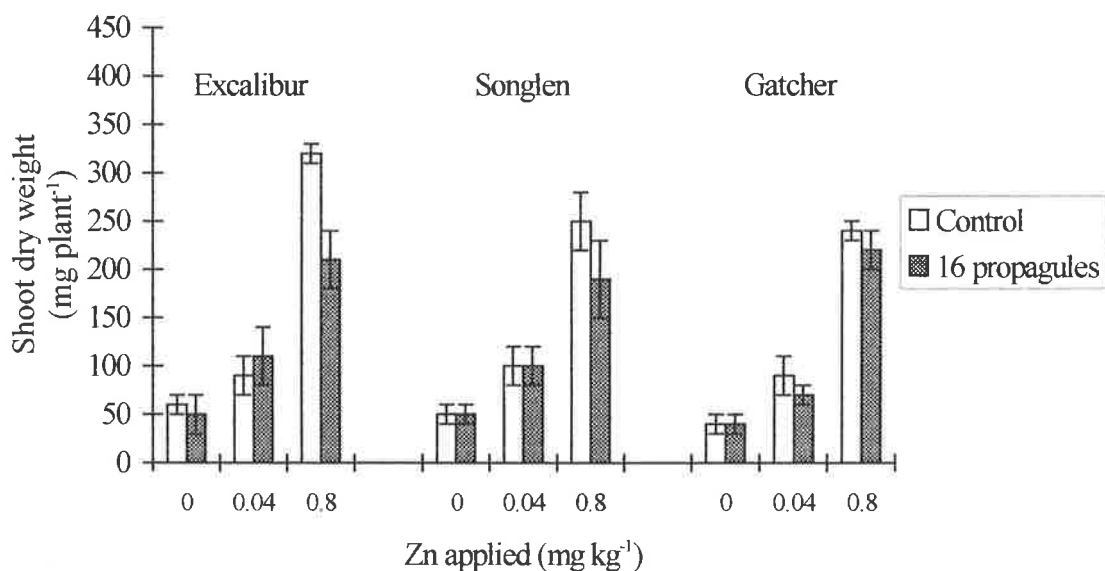


Figure 7.1. Shoot dry weight (mg plant⁻¹) of three wheat cultivars grown for 28 days in soil fertilised with three Zn rates with or without *Rhizoctonia* infection. Values are means of four replicates $\pm$ SE. Probabilities of treatments significantly influencing shoot dry weight: cultivar, $P = 0.03$; zinc, $P = 0.0001$, *Rhizoctonia*, $P = 0.05$; zinc $\times$ *Rhizoctonia*, $P = 0.0009$. All other treatment interactions were not significant at the $\alpha = 0.05$ level.

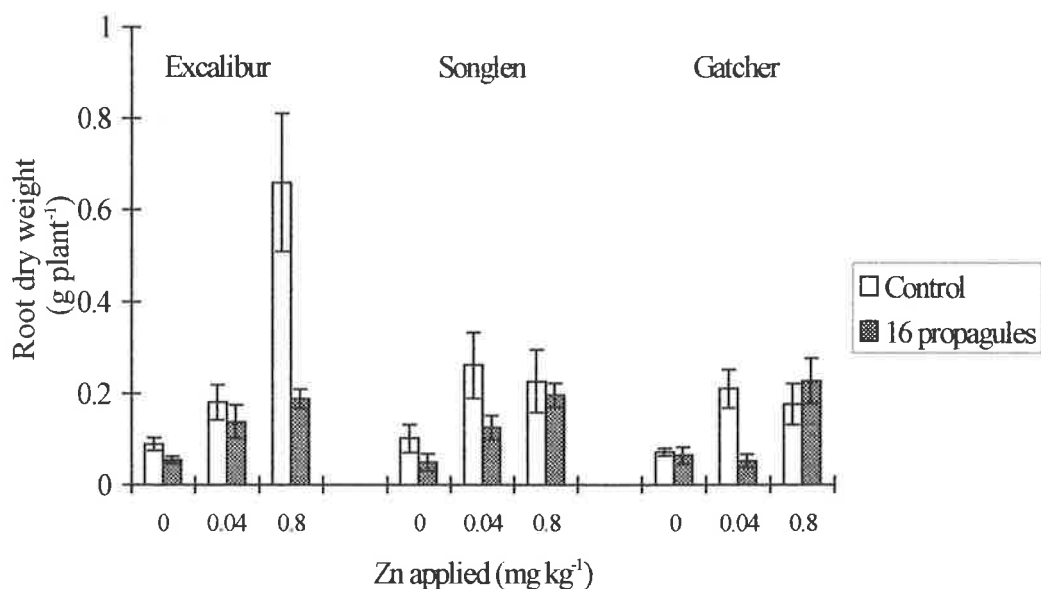


Figure 7.2. Root dry weight (mg plant⁻¹) of three wheat cultivars grown for 28 days in soil fertilised with three Zn rates with or without *Rhizoctonia* infection. Values are means of four replicates $\pm$ SE. Probabilities of treatments significantly influencing root dry weight were not significant at the $\alpha = 0.05$ level.

Root dry weight measurements indicated all three wheat cultivars obtained a similar root mass after 28 days growth except in one instance (Excalibur, 0.8 mg Zn kg⁻¹). Excalibur produced a large amount of roots in control plants when grown with an adequate Zn supply (Figure 7.2). On the whole, *R. solani* had a minimal effect on root dry weight of cultivars grown with an adequate Zn supply, except for Excalibur (Figure 7.2).

Zn status

Zinc concentration of shoots was significantly affected by soil-applied Zn and by *R. solani* treatment (Figure 7.3). *Rhizoctonia solani* inoculum decreased Zn concentration of all plants only at the adequate supply of Zn. The soil application rates of 0 and 0.04 mg Zn kg⁻¹ were deficient for optimum growth.

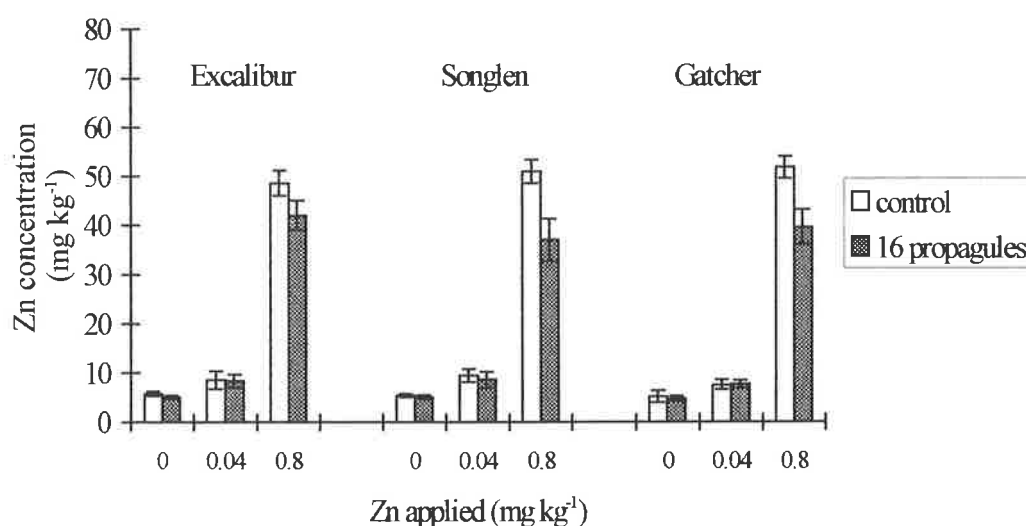


Figure 7.3. Zinc concentration (mg kg⁻¹) in shoots of wheat grown with three levels of soil Zn with or without *R. solani* inoculation 28 days after sowing. Values are means of four replicates $\pm$ SE. Probabilities of treatments significantly influencing Zn concentration: cultivar, $P = 0.068$; Zn treatment and *Rhizoctonia*, $P = 0.0001$. Zinc $\times$ *Rhizoctonia*, $P = 0.0001$. All other treatment interactions were not significant at the $\alpha = 0.05$ level.

Zinc transport efficiency of cultivars was similar when grown without added Zn. Zinc-adequate plants, however, showed a large difference in their responses. Zinc transport efficiency of Songlen and Gatcher was more than double that of Excalibur when grown in the absence of *R. solani* (Figure 7.4). Gatcher had a higher Zn transport efficiency than Songlen when not infected. However when plants were infected with *R. solani* Excalibur demonstrated a higher Zn transport efficiency than both Songlen and Gatcher.

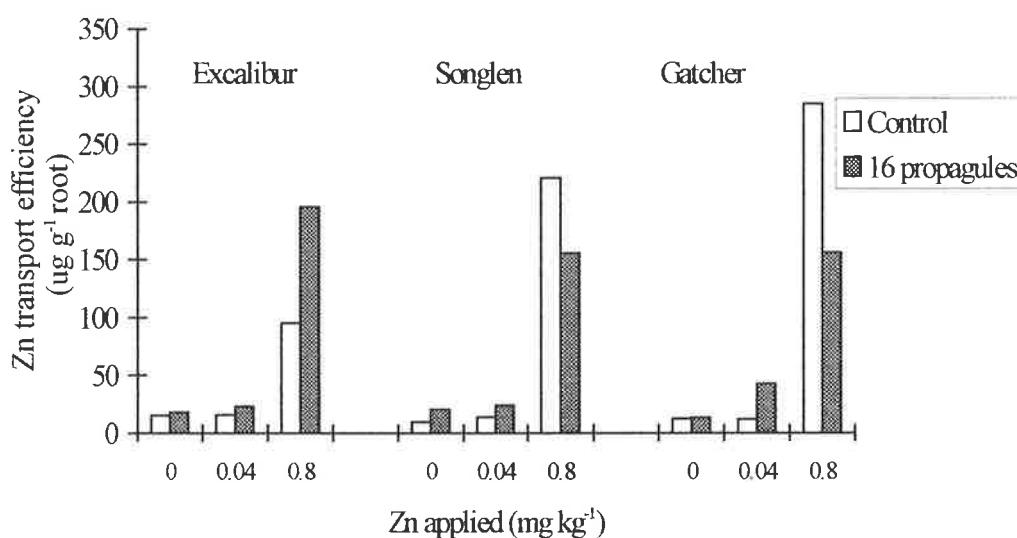


Figure 7.4. Zinc transport efficiency ($\mu\text{g g}^{-1}$ root) in shoots of wheat grown with three levels of soil Zn with or without *R. solani* inoculation 28 days after sowing. Values are means of four replicates.

7.4 Discussion

Tolerance of wheat to *R. solani* has been identified in the present study.

Tolerance is defined as the maintenance of growth in the presence of disease (*Rhizoctonia* tolerance). Furthermore this tolerance may be related to the Zn transport efficiency of control plants grown in Zn-adequate soil.

Gatcher was able to sustain dry matter production over all Zn treatments in the presence of the pathogen. Hence, Gatcher is classified as more tolerant to *Rhizoctonia solani* than either Excalibur or Songlen, which suffered greater yield loss in the presence of the disease at adequate Zn. It is interesting to note that Excalibur attained greater shoot dry matter production than either Songlen or Gatcher when no Zn and adequate Zn was supplied. Infection of Excalibur by *R. solani* under Zn-adequate conditions resulted in a greater loss of shoot dry matter than either in Songlen or Gatcher. The yield of all three cultivars grown with adequate Zn and infected with *R. solani* was similar. The greater proportional loss of shoot dry matter of Excalibur under conditions of adequate Zn and *R. solani* infection therefore appears to be a consequence of the increased yield of Excalibur over the other cultivars tested in control plants supplied with adequate Zn.

As disease is not uniformly distributed in a field or of similar intensity each year it may be beneficial to select Excalibur for current farming practice, as it has the highest uninfected yield. However, from a plant breeding point of view Gatcher may be better to select for further breeding programmes for tolerance to *R. solani*, as it demonstrates a lower loss in yield owing to *R. solani* infection. This chapter further demonstrates the difficulty in evaluating disease impact and nutrient relationships.

Results indicated that increasing Zn fertilisation enhanced the Zn status of the wheat plants (Figure 7.3). Zinc-sufficient plants produced higher shoot and root dry matter (Figure 7.1 and 7.2) than their Zn-deficient counterparts. Zn concentrations in the insufficient treatments (0 and 0.04 mg kg⁻¹ applied Zn) were lower than that required for adequate plant growth and were similar in all three cultivars (Figure 7.3).

Increased tolerance to *R. solani* was not connected with Zn sufficiency but with the Zn transport efficiency of the cultivar (Figure 7.1 and 7.4). Gatcher had the greatest Zn

transport efficiency at 0.04 mg kg^{-1} applied Zn of the three wheat cultivars tested and also the least yield loss in the presence of *Rhizoctonia*. Excalibur is transporting more Zn to shoots than Gatcher when infected and grown at the highest Zn level. The same tendency appears when grown at the lowest Zn level. The three wheat cultivars differed markedly in their Zn uptake rate (Figure 7.4). The uninfected plants from the two lowest Zn treatments had Zn transport efficiencies of $20 \mu\text{g g}^{-1}$ root in all three cultivars. These values were all quite low but the three cultivars behaved differently in terms of the Zn deficiency symptoms and yield. Gatcher and Songlen showed severe Zn deficiency symptoms 8 days before Excalibur. Despite Excalibur having shoot Zn concentrations similar to Gatcher and Songlen, it showed Zn deficiency symptoms later and its shoot dry matter was significantly more than Gatcher and Songlen at 0.04 mg kg^{-1} applied Zn. These results indicate that Excalibur is more Zn efficient compared to Gatcher and Songlen. Excalibur has also been documented as Zn-efficient under field conditions (Graham *et al.*, 1992).

It is clear from the present study that Zn does not directly suppress *Rhizoctonia* root rot disease, but tolerance is increased owing to physiological effects of Zn on the cereal plant. It is hypothesised here that the increased tolerance to *Rhizoctonia* is due to a differential allocation of photosynthates (Figure 7.1 and 7.2) in different cultivars, hence differences in root morphology and architecture will arise between different cultivars.

Inhibition of shoot growth but continuation or even enhancement of root growth under root disease might reflect alteration in photosynthate allocation.

Kirkegaard *et al.* (1999) present results that challenge the conventional 'supply' explanation for reduced seedling growth caused by root-rotting fungi. The results of the study presented here also challenge the 'supply' explanation but disagree with Kirkegaard *et al.* (1999) in other respects. Kirkegaard *et al.* (1999) present results indicating that the reduced shoot growth of *Rhizoctonia*-infected wheat seedlings cannot be overcome by increasing the supply of water or phosphorus to the leaves. The study presented here demonstrates that supply of Zn to the plant root environment is not the limiting factor that caused yield loss but rather the lowered Zn transport efficiency that limits the yield. This

leads to the hypothesis that cultivars of wheat with higher nutrient uptake rates will be more tolerant to *Rhizoctonia solani*.

A better understanding of the physiology of plant/pathogen interaction may provide clues to reduce the impact of the disease on early leaf growth. Further experimentation needs to be conducted with respect to disease, Zn, photosynthetic rate, photosynthate allocation and tolerance.

Chapter 8

General Discussion

Plants growing in natural and agricultural ecosystems are exposed to a range of abiotic and biotic stresses during development and growth. How a stress affects a plant depends on the nature of the stress. Stress can be distinguished as (1) being caused by growth in an environment with insufficient resources for the support of maximal growth and (2) being caused by direct physiological damage to the plant resulting in disruption of metabolism.

Infection of plants by fungal pathogens commonly results in a stress being applied to the plant by direct physiological damage to the plant organs. This is observed in fungal pathogens which attack the leaves, such as *Magnaporthe grisea* (rice blast fungus) and *Colletotrichum graminicola* (anthracnose) and other fungi which attack the plant stem and root system such as *Gaeumannomyces graminis* (take all), *Fusarium sp.* (rot) and *Rhizoctonia solani* (bare patch).

Control of many plant pathogenic fungi is achieved through the incorporation of genes for resistance or tolerance in the host plant or through the application of fungicidal compounds. Given the decreasing acceptability of wide spread fungicide use and increasing incidence of fungicide tolerance in fungal pathogens as well as a lack of a suitable crop rotation to reduce *R. solani* (MacNish, 1986) there is a need to explore resistance and tolerance to the pathogen as a method of control.

Disease resistance in plants is an observed outcome of the interaction between a pathogen and its potential host, and represents the physiological state in which the pathogen fails to establish itself on a putative host. Resistance can be the result of a number of distinct mechanisms working either together or separately. The relative lack of information on plant resistance to soil-borne pathogens has resulted in numerous

hypotheses to explain the mechanisms of disease resistance and in particular the role of nutrition in cereal crop resistance to diseases. Tolerance of the host plant to pathogen attack is measured in terms of its ability to maintain its own growth or yield in spite of infection by a plant pathogen.

This thesis addresses the interaction of *Rhizoctonia solani* with wheat. *Rhizoctonia solani* is a fungal root pathogen that causes rotting of the root system of many plant species leading to the eventual severance of the roots. In Australia, reduced yields associated with the adoption of minimum tillage practices has been attributed to increased incidence of *R. solani* infection. In the absence of effective sources of cultivar resistance to *R. solani* (Neate, 1989) and a lack of a suitable crop rotation for control, methods for improving tolerance of cereals to the pathogen need to be addressed.

Tolerance to *R. solani* has previously been observed in both barley and oat cultivars (McDonald and Rovira, 1985). However, screens of 9 wheat cultivars for tolerance to *R. solani* did not reveal sufficient variability to warrant further screening of breeding lines (Neate, 1989). Whilst differences in *R. solani* tolerance between cultivars within cereal crops are neither great enough nor consistent enough to be recommended as a method of control of *R. solani* in the field (Neate, 1989), the experimental conditions of previous screens for tolerance (Neate, 1989) did not include nutrient-deficient conditions. It has been demonstrated (Grewal, 1996; Thongbai, 1993b) that an apparent tolerance of a host to a pathogen is evident when one studies the system under nutrient limiting conditions. As *R. solani* disease of crop plants in South Australia is generally observed in low rainfall areas on alkaline soils displaying various nutrient deficiencies, further investigation of tolerance to the disease in nutrient limiting conditions is required.

Whilst conjecture remains over the mechanisms acting to ensure tolerance, it is apparent that tolerance of wheat to *R. solani* is a complex process involving the association of the abiotic and biotic environment, the pathogen population and virulence, and the vigour and developmental stage of growth of the host plant. Changes in environmental conditions which are either beneficial to the growth of the host plant or detrimental to the growth of the pathogen are likely to decrease disease severity and or incidence.

Conversely plants growing in unfavourable conditions are more likely to display greater susceptibility to infection. However, environmental conditions which either increase plant growth or decrease pathogen pathogenicity do not necessarily cause a decrease in disease severity and incidence. It is possible that such an environmental change which has an adverse effect on the pathogen, may increase disease incidence (Katan, 1996). Hayman (1969) showed that although the growth of *Rhizoctonia solani* was reduced at low temperature, damping-off disease incidence in cotton caused by this pathogen was higher at low temperatures than at higher ones. This was attributed to increased seed exudation at low temperatures, which allowed increased pathogenesis. The herbicide diphenamid increased disease incidence of *Rhizoctonia solani* in pepper, in spite of the fact that it is slightly toxic to the pathogen (Katan and Eshel, 1974). This occurred because the herbicide was more harmful to the other soil microorganisms than it was to the pathogen (Katan, 1996).

One of the major environmental factors that may influence disease severity and incidence is the availability of nutrients to the plant. It is known that well nourished plants suffer less yield loss than plants of poorer nutrient status when subject to infection by plant pathogens (Garrett, 1970). When studying the effect of a root rotting pathogen such as *R. solani*, this phenomenon may simply be due to well-nourished plants producing more roots and consequently "escaping" from fungal attack, rather than roots of well-nourished plants being more resistant to disease. Garrett (1970) supports the hypothesis of nutrient deficiency as a factor predisposing the host to infection, namely that "Any nutrient deficiency, if sufficiently severe, will seriously impair host resistance to infection; correction of the deficiency will control the disease and so infection by one or more fungal pathogens is the result of an unhealthy condition rather than the primary cause of it". Alternatively nutrient deficiency may be the result of infection limiting the ability of the plant to take up nutrients.

Bloom *et al.* (1985) hypothesised that plants respond to insufficient resources by changes to the growth of plant organs to maximise acquisition of those limiting resources. If *R. solani* infection is the cause of nutrient deficiency because the pathogen causes

severance of the roots thereby limiting the organs required for nutrient uptake, tolerance to the disease would arise from the ability of the plant to maintain the growth of roots despite infection by *R. solani*. It is apparent that tolerance of wheat to *R. solani* infection may arise through an interaction of both phenomena described above. Tolerance in nutrient efficient cultivars may arise because their improved nutrient status renders them less susceptible to *R. solani* infection. In addition improved nutrient status may allow more vigorous growth of roots required for nutrient uptake inspite of *R. solani* infection.

Although few diseases can be totally eliminated by any specific nutrient element, the severity of most diseases can be greatly reduced by proper nutrition (Huber and Graham, 1998). Knowledge of the relationship of plant nutrition to disease provides a basis for modifying current agricultural practices to reduce disease severity in integrated crop production systems. Rye is one of the most efficient cereal plants for nutrient absorption (Graham, 1984, 1988; Cooper *et al.*, 1988) and is considered resistant to take-all because of its rapid response to infection by “walling-off” penetration hypha of pathogen *Gaeumannomyces graminis* (Huber and Graham, 1998). Genotypes of triticale, the interspecific cross of rye with wheat, which possess the nutrient uptake efficiency of rye are also resistant to take-all, while triticale lines missing this characteristic are as susceptible as the wheat parent (Huber and Graham, 1998).

Thongbai *et al.* (1993b) demonstrated that Zn sufficiency of wheat suppressed disease severity of *R. solani*. A potential link between Zn nutrition and *R. solani* infection in wheat was therefore established.

The results presented in this thesis demonstrate that the interaction between Zn nutritional status of plants and the corresponding tolerance to *R. solani* is an extremely complex system. The effect of Zn on tolerance to *R. solani* is likely to involve the interaction of such factors as Zn-induced changes in root morphology and a direct physiological role of Zn in maintaining high yields.

There is no clear evidence to explain how zinc suppresses certain diseases. It has been hypothesised that enhanced membrane integrity in Zn-sufficient plants renders them less prone to infection by *R. solani* (Graham and Webb, 1991). Welch *et al.* (1982) have

suggested that an external supply of Zn in the environment surrounding the root is required as Zn may be important in structural and functional integrity of the root-cell plasma membrane, a role that was confirmed by Cakmak and Marschner (1988a) and Welch and Norvell (1993). Owing to the observation that Zn-deficient roots have leaky membranes, it is proposed that organic substances leaking from Zn-deficient membranes may be substrates for, or be chemotactic to, root pathogens. In one specific report organic acids, amino acids, and phytosiderophores were found to be released from Zn-deficient roots (Zhang *et al.*, 1989).

In wheat, when Zn was supplied to only half of a vertically split root system, root growth was depressed in the unsupplied half without affecting shoot growth (Robson and Snowball, 1989, 1990). In the experiments presented here a similar observation was made where significant reductions in root length were noted in response to Zn deficiency, however shoot dry matter was not significantly reduced. Such a result indicates Zn deficiency impairs fibrous root development. On the basis of this set of results it is suggested *R. solani* can infect Zn-adequate plants to a greater extent owing to the increased fibrous root system. However despite an increase in *R. solani* infection, Zn-adequate plants displayed higher tolerance to *R. solani*. It is the identification of the mechanism which allows this increased tolerance under conditions of increased infection which may provide the basis for novel control of the pathogen. The mechanism for increased tolerance may be reliant on several factors including the ability of the plant to extract Zn from the surrounding soil environment, the ability to efficiently transport Zn within the plant and the efficiency which Zn is utilised in metabolic reactions.

Data from this study suggests that the actual availability of Zn either from the soil or from the seed has little effect on tolerance to *R. solani*. That is plants displaying a high susceptibility to *R. solani* infection do not increase tolerance to the pathogen in response to either applied Zn or increased Zn content in the seed. This is in direct contrast with Thongbai *et al.* (1993b).

Nutrient uptake by the plant is likely to be extremely sensitive to changes in root radius (Darrah, 1993). A four fold increase in radius was predicted to increase P uptake in

corn almost three fold (Silberbush and Barber, 1983), and considerable differences in root diameter have been demonstrated to exist within and between species, with diameters ranging from 25 μm to > 500 μm (Fitter, 1991). It is possible that considerable differences in root diameters exist between cultivars of wheat and that this is one factor that needs to be considered when screening for tolerance to a root pathogen. Measuring root diameters with the availability of high-resolution scanners and appropriate computer software is not an extremely laborious task and one that is readily available.

The capacity to take up Zn from the soil may also be a function of an inducible Zn uptake system whereby limiting Zn in the surrounding environment induces a plant mechanism to increase Zn uptake. This may take the form of an increase in fine root production as demonstrated in Chapters 5 and 6. Root pathogens reduce the functional length of roots. The effect of root pathogens on root system form and architecture is currently impossible to predict (Darrah, 1993). However the possible importance of root length for efficient nutrient capture and disease escape responses, root architecture requires more attention. Root architecture is both genetically controlled and environmentally induced (Barber and Silberbush, 1984; Fitter, 1991), and as such incorporation of traits controlling root architecture into a given cultivar through conventional breeding programs appears difficult. However, if the genetic basis of the factors controlling root architecture can be identified, the variation in root architecture may be utilised in future breeding programs aimed specifically at the production of nutrient-efficient, disease-tolerant cultivars.

Translocation of Zn within the plant has also been demonstrated to affect the relative tolerance to *R. solani* infection. Little is known about the transport of Zn within the plant however several factors have been observed to have an effect. The amount of Zn translocated to the leaves of seedlings may be heavily dependent on the Zn content of the seed from which the seedling arose as Zn is readily mobilised from seed reserves (Longnecker and Robson, 1993). The current study however suggests that, whilst the Zn content of seed may be a determinant of Zn content in the growing seedling the amount of Zn in wheat seeds had no significant effect on *R. solani* infection of the developing

seedling. Other factors which effect Zn translocation, such as availability of Zn in the surrounding environment, and the mechanism of Zn ion transport need to be addressed. Pearson and Rengel (1995) demonstrated that Zn accumulates in the older leaves of seedlings grown under low Zn conditions. The accumulation of Zn in the older leaves may cause Zn to become limiting to the developing root system thereby decreasing the ability of the plant to tolerate *R. solani* infection.

The decreased tolerance of plants grown in Zn-limiting conditions is likely to be caused by a limitation in Zn required for several metabolic reactions involved in both plant defence and normal growth. The association of active oxygen species with the induction of defence responses to plant pathogens has been demonstrated in numerous plant-pathogen interactions (Sutherland, 1991). The active oxygen produced in response to pathogens has been hypothesised (Baker and Orlandi, 1995) to have direct antimicrobial effects and to play a role in other defence mechanisms including lignin production, lipid peroxidation and phytoalexin production. Superoxide generation by NADPH-preferring oxidases has been identified as part of the cellular defence or resistance response in plants (Cakmak and Marschner, 1988a). However, it is still unclear which mechanisms predominate during plant/pathogen interactions. NADPH oxidase has been located in the plasma membrane of potato tuber cells (Doke and Chai, 1985). In this particular case, plasma membrane-bound NADPH oxidase was suggested to generate superoxide radicals upon infection of potato tubers with incompatible races of *Phytophthora infestans* (Doke, 1985). Vera-Estrella *et al.* (1992) observed increases in active oxygen species, when tomato was infected with *Cladosporium fulvum*, which suggested that changes in the redox processes at the plasma membrane may be involved in the plant defence response against pathogens. Utilisation of NADPH by oxidases is strongly impaired by Zn^{2+} ions (Chvapil, 1979; Jeffery, 1983). If this is the situation, then increased plant nutritional status would be expected not to suppress disease as oxygen free radicals would be of lower availability for the destruction of invading pathogens. However, zinc-deficient plants have been shown to be more susceptible to disease than Zn-adequate plants. The observation that plants grown under marginal Zn deficiency conditions have less infection than plants

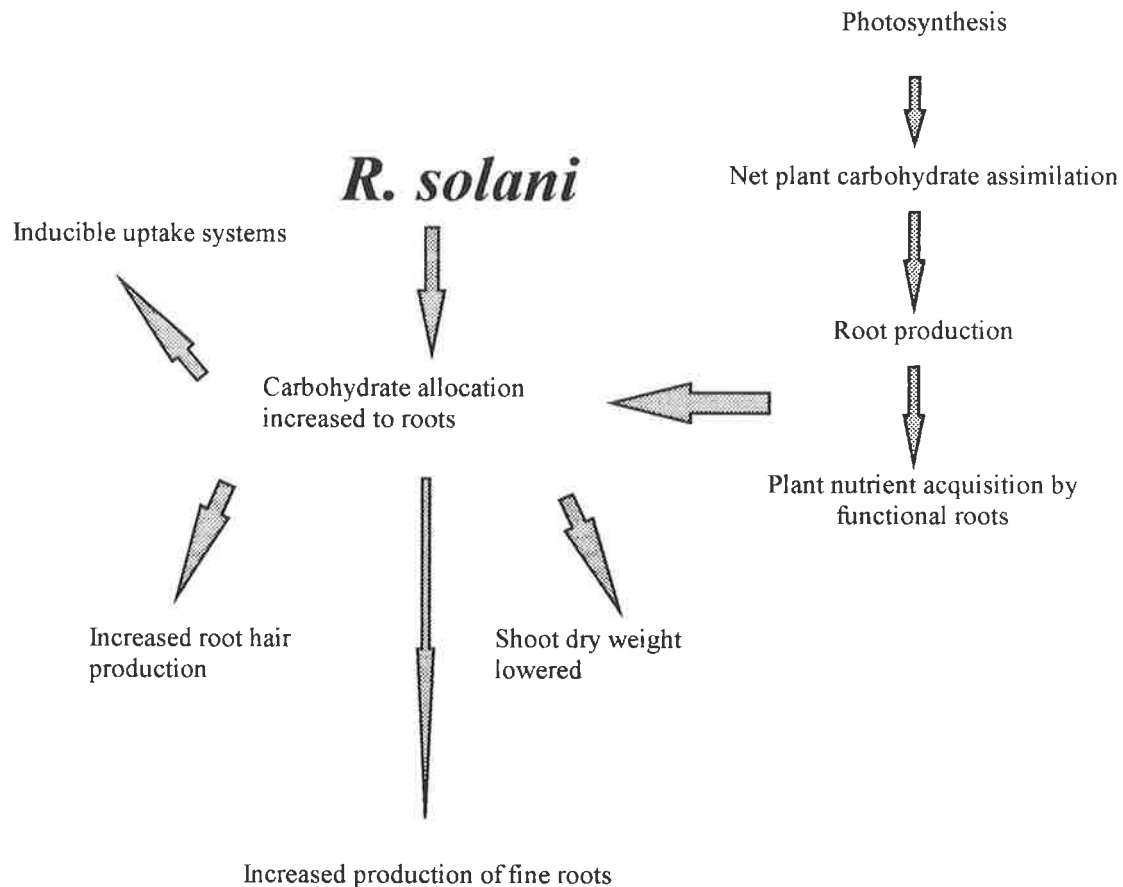
grown in adequate Zn conditions (Thongbai *et al.*, 1993b) may indicate that there is a balance between the number of toxic oxygen free radicals and membrane integrity.

Whilst the Zn content of seeds does not appear to affect tolerance to *R. solani* infection, it is possible that the content of carbohydrates or other nutrients in the seed does affect tolerance. In very young plants root carbohydrates can originate from seed reserves as well as from current photosynthesis, but the seed source becomes of lesser importance as the plant develops. After the seedling stage there is therefore a close relationship between root growth and photosynthesis (Van den Driessche, 1987). It is proposed that seeds with higher carbohydrate and nutrient content are possibly able to 'escape' the disease compared to their counterparts originating from seed with a lower carbohydrate nutrient content.

Depending on the developmental stage of a plant an average of 25-50% of the carbohydrates produced per day is allocated to the roots for growth, maintenance and other functions such as ion uptake (Marschner, 1995). Owing to an increase in root length in parallel to a decrease in yield in the presence of *R. solani*, it is suggested that in *Rhizoctonia*-infected plants the roots become the dominant sink for carbohydrates. Therefore seeds with a higher carbohydrate content, and seedlings with an increased photosynthetic capacity may display increased tolerance to *R. solani* by virtue of increased carbohydrate availability. Here it is proposed that the effect of Zn nutrition on *R. solani* infection can be associated with the plant disease 'escape' theory put forward by Huber (1981) (see Figure 1).

Whilst the preliminary characterisation of the Zn-*Rhizoctonia* interaction has been completed, the actual mechanism of disease tolerance remains speculative until a cause for reduced yield of the host in the presence of the pathogen is identified. Kirkegaard *et al.* (1999) propose that the reduction in leaf growth due to *R. solani* infection is not caused by root rot reducing the water or nutrient supply as commonly supposed, but is a response of the roots of wheat that produce inhibitory signals that travel in xylem sap and reduce leaf growth. If this is the case, it is possible that an adequate Zn nutritional status decreases either the amount of inhibitory signal or the translocation efficiency of that signal.

Figure 1. Conceptual model depicting the influence of *Rhizoctonia solani* on whole-plant structure and root proliferation.



Alternatively increased Zn nutritional status may cause the exudation of specific chemicals into the surrounding rhizosphere. These may act as chemotactic signals to numerous soil bacteria. Such bacteria may be effective as antagonists to weak soil-borne plant pathogens (Howie and Echandi, 1983). *Bacillus megaterium* strain B153-2-2, is a root coloniser and potential biocontrol agent for *Rhizoctonia* root rot of soybeans (Zheng and Sinclair, 1996). The availability of microbes which can influence the interaction of a plant and a pathogen is also affected by management practices (Bolton *et al.*, 1985). The ecology of the root-microbe interactions after minimum tillage practices is vastly different from the one after extensive ripping used to prepare the seedbed. The changes in the soil physical and

chemical properties resulting from tillage greatly alter the soil matrix supporting growth of the microbial population (Kennedy and Smith, 1995). In no-till agricultural systems, microbial activities drastically differed with depth, with the greatest microbial activity occurring near the no-till surface, while in the tilled system, activities were more evenly distributed throughout the ripped layer (Doran, 1980). The observed increase in *R. solani* infection in no till systems may therefore be in part due to changes in soil microbial activities.

It is proposed that before differences in tolerance to *R. solani* in wheat cultivars are consistently established, the nutrient uptake efficiency of the cereals needs to be vastly improved. It was demonstrated in Chapter 6 that the mechanism of Zn efficiency (as defined by Graham *et al.* (1992)) was disrupted by *R. solani* and tolerance of *R. solani* was related to the ability of the diseased plant to maintain Zn uptake despite the infection. It is concluded that root morphology is not in all cases associated with tolerance to *R. solani* infection (Chapter 5) but is associated with increased Zn uptake and furthermore directly related to the Zn transport efficiency of the cultivar (Chapter 7). Despite the importance of Zn as a micronutrient for plant growth, there have been relatively few studies of the mechanism of Zn uptake and transport. This study clearly shows that an understanding of nutrient uptake and efficiency is needed before the effect of nutrition on differences in tolerance of wheat cultivars to *R. solani* can be established.

Harmens *et al.* (1993) suggest Zn-efficient plants have a more effective sequestration mechanism inside their root cells owing to the direct effects of Zn on root growth, and higher Zn concentrations in the roots of Zn-efficient *Silene vulgaris* when grown with an adequate Zn supply. As root Zn concentrations were not determined in this study, the role of Zn sequestration in root cells on Zn uptake mechanisms and tolerance to *R. solani* cannot be ascertained. However, the translocation of Zn from roots to shoots appears to be an adequate indicator of Zn uptake in this type of study as it is the yield of plants that indicates tolerance to *R. solani* infection. Rengel and Graham (1996) demonstrated that net Zn uptake per unit of root is lower in plants pre-grown with sufficient Zn indicating the importance of internal Zn concentration in regulating Zn

uptake. Experiments in this thesis indicate that *R. solani* does reduce shoot Zn concentration (but not below critical), therefore it can be established that plants continue to take up Zn inspite of direct damage by *R. solani* infection on roots. Zinc uptake has been shown to increase with increasing Zn application (Dong *et al.*, 1995) indicating plant growth is dependent on root functions. As infection by *R. solani* has been shown in this study to have dramatic effects on root growth and development, it is suggested that greater Zn uptake and transport efficiency contributes to plant tolerance of *R. solani*.

Mineral nutrients, whilst having a direct effect on plant growth, appear to have a more indirect effect on disease by offsetting reduced absorption of nutrients, stimulating new root growth, overcoming nutrient sink effects, altering host resistance or changing the soil microflora (Huber, 1981). Another option for plant breeding, for increased tolerance of soil-borne diseases, may be in the development of cereal cultivars with longer finer root systems. In this way a plant with an inherently longer root system will be more capable of outgrowing the disease in question than their counterparts with a shorter root system. Longer and thinner roots will also benefit the uptake of other nutrients, especially those that are diffusion-supplied (Dong *et al.*, 1995). Branching of the root system may also be an important consideration for the plant breeder. However there may be some stimulation of branching caused by the disease, and genetic differences may only be apparent in infected plants.

The observation that adequately nourished plants have increased tolerance to *R. solani* could also be explained by increased vigour. In this case, increased susceptibility to *R. solani* might cause some kind of environmental stress imposed on the plant resulting in increased infection. The dependence of disease resistance in plants on other limiting factors is accepted by plant pathologists (Van der Plank, 1975).

The practical implication of this study is that, with judicious use of fertiliser in nutrient-deficient areas where *R. solani* is prevalent, sustained wheat production can be achieved by reducing the severity of the disease through increasing plant vigour via improved nutrition. It is observed that a reduction in disease severity with the application of a limiting nutrient generally results from increased plant tolerance through altered

metabolic pathways, plant constituents or exudates and not from a direct effect of the nutrient itself.

The effort to protect plants from pathogens using nonchemical means is critical. Even where chemical pest control has been cost-effective, agricultural-scale use is becoming socially undesirable. Increased efforts in the area of nonchemical pathogen control are both urgently warranted and necessary to sustain agricultural production at present levels.

Without *Rhizoctonia*-resistant cultivars existing, tolerance to the disease is the next major step in controlling the effects of the pathogen. The research described here should provide the basis for a more detailed analysis of cultivar tolerance to *R. solani* in *T. aestivum*. This may be by further investigation into root morphology and researching chemical stimuli in an attempt to investigate the reduction in yield.

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Appendix 1

The saprophytic growth of *R. solani* in differing P concentrations

Introduction

The addition of a particular micronutrient to deficient soil may affect the status of the plant, the pathogen, or both, thus affecting the host-pathogen relationship. Previous experimentation has indicated that the application of Zn to Zn-deficient soil reduces the incidence of *Rhizoctonia solani* (Thongbai *et al.*, 1993b). The mechanism by which Zn operates to reduce the level of *R. solani* infection is unclear. Results of Thongbai *et al.* (1993c) indicate that Zn has no effect on the saprophytic growth of the fungus growing in soil or on synthetic medium. These results indicate that Zn does not have a direct effect on the growth of the fungus, therefore the reduced incidence of the disease in the presence of Zn must be a result of applied Zn interacting directly with the plant.

Results obtained in Chapter 5 demonstrate adequate soil-applied P increased plant tolerance to *R. solani*; however, an increase in the number of spear tips and infected root lengths of P-adequate plants was evident when compared to P-deficient plants.

It is hypothesised that P may affect the saprophytic growth of the fungus, resulting in increased saprophytic growth and virulence of the fungus with higher levels of applied P.

Aim

The aim of the experiment was to investigate if differing P supply effected the saprophytic growth of *R. solani* on synthetic media.

Experimental design

Rhizoctonia solani (AG-8) was grown on synthetic agar (1/6 NDY- Appendix 2), with seven different levels of added P (Table 1). The experiment was arranged in a randomised design with four replicates.

Agar medium

The synthetic agar used in this experiment was a weak Czapek-Dox medium with added yeast extract (1/6 NDY-Appendix 2). The agar was prepared using Davis agar (Oxoid agar) and DD water. Fourteen mls was put into each of 28 small vials.

P solution

Phosphorus solutions of seven concentrations, according to the treatments outlined in Table 1, were prepared from a stock solution of KH_2PO_4 and DD water.

Table 1. P concentration contained in 15 ml of agar.

P treatment ($\mu\text{g (15 ml)}^{-1}$ agar)
0
7.5
22.4
37.5
56.1
75
187.5

The plates were incubated at 20 °C for 5 days and colony diameter was measured on three days (days 2, 3 and 5) during this period of growth.

Procedure

Twenty eight vials of prepared agar medium plus 1 ml of P solution were placed in a 45 °C water bath to melt agar so as not to inactivate the Steptomycin and Tetracyclin. Once the contents of the vials had become viscous they were transferred to a laminar flow cabinet before being poured into plastic petri dishes (9 cm diameter). They were left to cool and set. Pure culture of *R. solani* was used as in the previous pot experiments. The fungus was cultured on 1/6 NDY agar, as previously described, in sterilised petri dishes at 20 °C for 4 days. Actively growing hyphae of *R. solani* were aseptically transferred to the centre of each 9-cm petri dish, then grown in the dark for five days. The colony diameters were measured at regular intervals using the average of four random diameter lines.

Results

Increasing the rate of applied-P had no effect on the rate of saprophytic growth of the fungus (Figure 1).

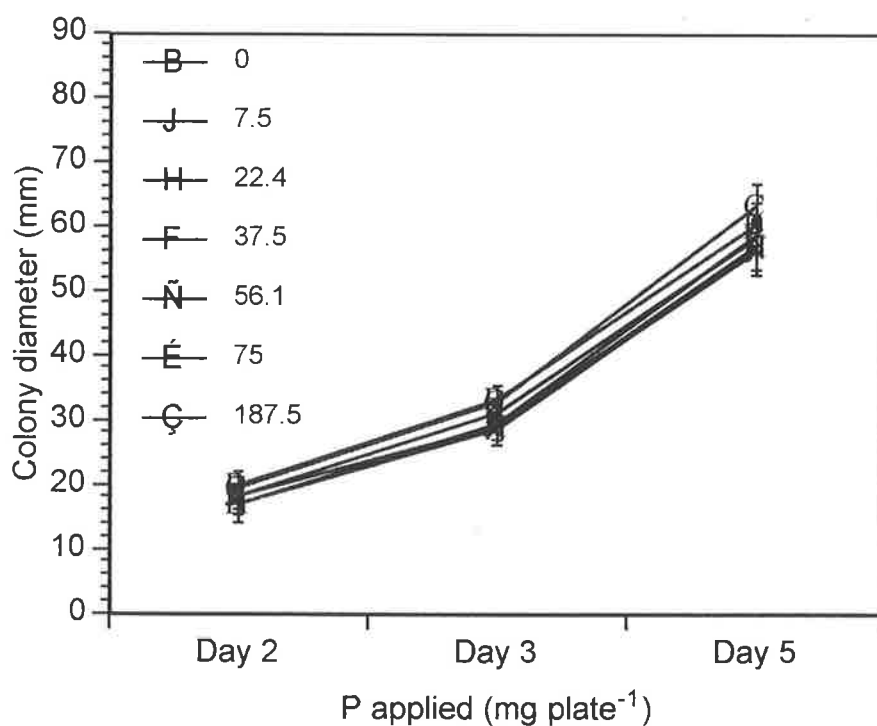


Figure 1.1. Growth of *R. solani* (AG8) at seven different rates of P on 1/6 NDY medium. The measurement of colony diameter was averaged over three replicates on days 2, 3 and 5

Conclusion

The final colony diameter of *R. solani* was not affected by increasing P. The highest level of P used in the pot experiment (Chapter 3) was only mg P/kg soil which in Petri dishes had no effect on increasing the growth of the fungus. Therefore it is suggested increased levels of applied P do not stimulate the saprophytic growth of *R. solani* and are not the cause of increased infection as seen in Chapter 3. The effect of applied P on increasing the visual symptoms of the disease but decreasing the yield loss caused by the disease must be a physiological effect on the plant and not on the fungus directly.

It is concluded that P deficiency does not directly influence the growth of *R. solani* as was also reported with Zn (Thongbai *et al.*, 1993c) nor does P sufficiency affect the growth of the fungus.

Appendix 2

Media used for growing *Rhizoctonia solani*

1/6 NDY (Warcup, 1955)

Ingredients

	g/L
1. NaNO ₃	0.33
2. KH ₂ PO ₄	0.16
3. MgSO ₄	0.083
4. KCl	0.083
5. FeSO ₄	0.0016
6. Yeast extract	0.083
7. Sucrose	5
8. Davis agar (Oxoid)	14
9. Streptomycin sulphate	100 µg ml ⁻¹
10. Tetracycline hydrochloride	50 µg ml ⁻¹

Procedure

Add all compounds 1 to 7 to 500 ml of DD water and dissolve.

Add agar to 500 ml of DD water, microwave until dissolved, then add to 1st solution

Autoclave for 20 min at 121 °C, 100 KPa, cool to touch (45-50 °C) then add 9 and 10.