THE INFLUENCE OF ZN NUTRITIONAL STATUS ON THE SEVERITY OF *RHIZOCTONIA* ROOT ROT OF CEREALS

Thesis

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DECLARATION

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

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ABSTRACT

Rhizoctonia root rot is a major obstacle for farmers adopting minimum tillage in wheat growing areas in Australia, as well as other parts of the world, with sandy soils because conventional tillage is the most effective means of controlling this disease. These soils have also been reported as Zn deficient, a condition aggravated by the reduction in use of superphosphate fertilizer containing some Zn impurity. The research objective was to investigate the relationship between Zn nutritional status in cereals and the severity of *Rhizoctonia* root rot in both natural and controlled conditions, and then to establish the causal link behind any observed correlation found in the first phase of the study.

The severity of a root rot disease of cereals, caused by *Rhizoctonia solani* Kühn, was inversely correlated to the Zn status of plants in field studies in 1989 and 1990. In 1989, a survey was conducted in a farmer's field in South Australia where Zn deficiency and disease were both widespread. Zn concentration in 'Spear' wheat plants at the 3-leaf to early tillering stage was negatively correlated with the severity of disease. Of ten trace elements analysed, the correlation with Zn was by far the most significant ($R^2=0.52^{**}$).

The effect of Zn applications and their residual value on disease severity was further studied in a long-term field experiment in 1989 and 1990 in a field to which Zn treatments had been applied in 1986. There was a decrease in the area of *Rhizoctonia* bare patch with increasing Zn application; the recommended rate of 2.5 kg Zn/ha reduced the area affected by bare patch from 42% to 21% of the total crop area while overcoming Zn deficiency and increasing grain yield from 1.0 to 2.8 t/ha. In 1990, fresh Zn application treatments were applied to trial plots designed for this purpose, in

order to compare the response with the older Zn treatments applied in 1986. The areas of bare patch in the older Zn treatments were approximately 5% greater than those in the fresh Zn treatments. The results are consistent with the hypothesis that Zn deficient plants are more susceptible to root rot caused by *R. solani.*

The inverse correlation, between plant Zn concentration and the severity of *Rhizoctonia* root rot in the field, was examined in two experiments in a growth chamber. In the first experiment, wheat (cv Songlen) was planted in a Zn deficient soil (Laffer sand) with and without Zn and combined factorially with different inoculum densities of *Rhizoctonia solani* anastomosis group 8. When Zn was added, the percentage of seminal roots infected was significantly lower compared to the treatments without added Zn, showing that Zn suppressed the disease. A subsequent factorial experiment of four inoculum densities and six Zn levels (0, 0.01, 0.04, 0.1, 0.4 and 6.0 mg Zn/kg soil) was conducted to investigate the Zn effect in more detail. Disease severity was markedly decreased by the higher Zn applications; the disease score dropped sharply between treatments of $Zn_{0.04}$ and $Zn_{0.1}$, a difference which mirrored the plant yield response to Zn.

In both experiments the Zn concentrations in shoots were significantly different only among Zn treatments, not among the inoculum treatments. This indicated that inoculum density or disease severity did not reduce Zn concentration in the plant. Thus, disease did not exaggerate Zn deficiency, but rather, Zn sufficiency suppressed disease severity. A causal link between Zn nutrition and *Rhizoctonia* root rot severity is thereby established for the first time.

To quantify the effect of Zn on growth of the pathogen, the fungus was grown both in agar media and in soil with 10 levels of Zn in each system. There was a significant effect on growth rate of the fungus by increasing Zn treatment only at the toxic end of the scale. It could be concluded from these results that Zn status at the low end of the range has little or no effect on fungal growth. Thus, the suppression of disease severity as illustrated from the previous experiment is probably through

physiological processes in the plant rather than an effect of Zn on the saprophytic vigour of the fungus in the soil. The effect of Zn deficiency on root cell membrane leakage and carbohydrate metabolism was hypothesized as the mechanisms of increasing the disease severity in the plant.

This study identifies a strong relationship between Zn nutritional status and severity of *Rhizoctonia* root rot in cereals, provides a basis for understanding the mechanisms of this effect, and points to an additional approach to control of the disease.

PUBLICATIONS

1. Thongbai P, Hannam R J, Graham R D and Webb M J 1993a Interaction between Zn nutritional status of cereals and *Rhizoctonia* root rot severity. I. Field observations. Plant Soil (in press).

2. Thongbai P, Graham R D, Neate S M and Webb M J 1993b Interaction between Zn nutritional status of cereals and *Rhizoctonia* root rot severity. II. Effect of Zn on disease severity of wheat under controlled conditions. Plant Soil (in press).

3. Thongbai P, Webb M J and Graham R D 1993c Zinc deficiency predisposes winter cereals to *Rhizoctonia* root rot. *In* Ed. A D Robson Zinc in Soils and Plants. Kluwer Acadamic Publishers, Dordrecht (in press).

THESIS INTRODUCTION

Some cereal growing areas in Australia and other parts of the world have sandy soils which are easily eroded under heavy tillage. Minimum tillage, in the form of direct-drilling, is recommended in these situations and is being increasingly adopted by farmers. However, these areas are reported to be severely affected by *Rhizoctonia* bare patch (Dubé *et al.*, 1987) and minimum tillage has been associated with an increase in disease severity (Rovira, 1986). This adverse situation in the Mallee areas of Victoria and South Australia was severe enough for the Wheat and Barley Research Committee of South Australia to organize a workshop in 1987. Scientists, district agronomists, farmers, representatives from funding bodies and agribusiness met to find solutions to the problem. Setting research priorities and calling for research projects on this disease was the outcome of that workshop. This thesis is the result of one of these research projects.

In these same areas, the soils are predominantly calcareous and/or siliceous sandy loams inherently low in Zn. The occurrence of Zn deficiency in the Mallee areas was suspected to be so frequent that a survey program was established by the Waite Agricultural Research Institute and the SA Department of Agriculture to investigate the situation. Results of the survey showed that Zn concentration in the youngest expanded leaf blades (YEBs) of cereal plants from 90% of the farms in this area was lower than the minimum desirable level of 20 mg/kg (Reuter & Robinson, 1986). It has been hypothesized that severity of Zn deficiency in these areas has been exacerbated by a reduction in the use of single superphosphate fertilizer which contained a significant amount of Zn as an impurity, owing to a preference for high analysis phosphatic fertilizers (Graham *et al.*, 1989).

Since these two perceived problems, an increase in *Rhizoctonia* bare patch and an increase in Zn deficiency, may have happened in the same region in the same time frame, it is possible that they may be linked causally. Fifty years ago, in the Mallee region of Victoria, Millikan (1938) observed, in farmers' fields where root diseases were severe, less disease in plots receiving 17-35 kg $ZnSO_4$/ha, and the Zn resulted in a 32% increase in yield. However, the significance of this report has been largely ignored.

Lately, more nutrient-disease interactions have been reported, for example, the interaction between take-all disease of cereals and manganese deficiency (Graham & Rovira, 1984) and between crown rot disease and Zn deficiency in wheat (Sparrow & Graham, 1988). Thus, it became timely to study in detail the interaction of Zn with *Rhizoctonia* root rot or bare patch disease in cereals. **The primary working hypothesis of this thesis is therefore that Zn deficiency decreases the resistance of cereal plants to the fungus, *Rhizoctonia solani*, predisposing the plants to an increase in bare patch disease.**

This research started in 1989 with the objectives:

(i) to investigate the existence of a relationship between Zn nutritional status in cereals and severity of *Rhizoctonia* root rot in both field and controlled conditions;

(ii) to establish the causal link behind any observed correlation; and

(iii) to examine this interaction closely over a range of Zn and inoculum levels in order to speculate on the nature of possible mechanism(s).

CHAPTER 1

LITERATURE REVIEW

PART I: ZINC

In terms of a possible essential role for zinc (Zn) in biological systems, it was first found to stimulate the growth of *Aspergillus niger* by a French scientist, Raulin in 1869. He was followed by Timiryazev, in 1872, who demonstrated effects of Zn, not only on the growth and development of maize but also on curing chlorosis in the plants. These discoveries are considered to be the inspiration for the science of the essential trace elements (Shkol´nik, 1984).

I.1. ZN AS AN ELEMENT

Because of its electronic configuration, Zn, together with Mn, Fe, Cu and Mo, is located in d block of the periodic table which means it has electrons in the d orbital. Zn, with its complete $3d^{10}\ 4S^2$ outer electron configuration, is the only non-transition element in this block and always shows an oxidation state of II in its compounds. Other properties of Zn are the following (Wedepohl, 1969-1978, cited by Chesworth, 1991) :

Elemental properties	Atomic number		30
	Atomic weight		65.28
	Electronic formula		$[Ar]\ 3d^{10}\ 4s^2$
	Isotopic abundances		^{64}Zn 48.9%, ^{66}Zn 27.8%, ^{68}Zn 18.6%, ^{70}Zn 0.6%
Chemical properties	Radii -	atomic (A°)	1.34
		ionic (C.N.)	0.60 (4), 0.74 (6)
		Covalent (A°)	1.34-1.46

Ionization potential (eV)	(I) 9.394; (II) 17.964
Electronegativity	1.6

I.2 ZN IN SOILS

The average Zn concentration in the Earth's crust is reported to be 80 mg/kg and the distribution in soils ranges from 10 to 300 mg Zn/kg, with an average of 50 mg Zn/kg (Harmsen & Vlek, 1985). Being classified as one of the chalcophiles or elements that have an affinity to sulphide (Allegre, 1982), the primary source of Zn in soil is the ferromagnesian mineral in sulphide form, sphalerite (ZnS) (Krauskopf, 1972). This mineral scatters throughout the mineral fraction of soils, as well as breaking down to release Zn(II) which is probably held in crystal lattices of clays to become very immobile, or it may be held by an exchange site and adsorbed to the solid surface (Lindsay, 1972b). The latter is believed to be the most common and mobile form of Zn in soil (Kabata-Pendias & Pendias, 1984).

I.2.1. Zn behaviour in soils

Adsorption is a major factor contributing to low concentrations of Zn in solution in Zn-deficient soils (Ellis & Knezek, 1972). Two different mechanisms of Zn adsorption are proposed; one in neutral and acid media related to cation exchange sites and the other in alkaline media that is considered to be chemisorption and is highly influenced by organic ligands (Lindsay, 1972a; Farrah & Pickering, 1977). Of the total Zn in soils, 24-63% is Zn hydroxide adsorbed on clay surfaces (Zyrin *et al.*, 1976, cited by Kabata-Pendias & Pendias, 1984) and strongly pH-dependent (McBride & Blasiak, 1979), 14-38% is associated with hydrous Fe and Al oxides, 1-20% are readily mobile fractions and 1.5-2.3% are organic complexes, respectively (Zyrin *et al.*, 1976, cited by Kabata-Pendias & Pendias, 1984). Zn is also reported to be associated with manganese (Norrish, 1975).

Soil pH (Anderson & Christensen, 1988), clay minerals (Reddy & Perkins, 1974; Shuman, 1975,1976; Kabata-Pendias, 1980; Puls & Bohn, 1988) and hydrous oxides are likely to be the most important factors controlling Zn solubility in soil, while organic complexing and precipitation of Zn as hydroxide, carbonate and sulphide compounds appear to be of much lesser importance (Abd-Elfattah & Wada, 1981). Soil pH has been shown to affect Zn adsorption, either by changing the number of the available adsorption sites or by changing the concentration of Zn species to be adsorbed (Barrow, 1986a,b). In acidic, light mineral soils, Zn is more readily mobile and available and can be lost by leaching (Norrish, 1975).

Zn is immobilized in soils rich in Ca and P, in well aerated soils with S compounds, and soils high in Ca-saturated minerals such as allophane, imogolite and montmorillonite, as well as hydrous oxides. Decrease in solubility and availability of Zn with increased Ca-saturation and increased amounts of P compounds in soils, is the result of adsorption and precipitation processes, as well as interactions between these elements. These factors have a very practical impact on the likelihood of Zn deficiency in plants (Kabata-Pendias & Pendias, 1984).

Soil organic matter can both mobilize and immobilize Zn in soils (Chiridchai & Ritchie, 1990) depending on conditions. Although Zn-organic complexes have been considered as a minor component of total soil Zn (Abd-Elfettah & Wada, 1981; Kabata-Pendias & Pendias, 1984; Hickey & Kittrick, 1984), they could have measurable impact on availability of Zn in soils and/or soil solutions with marginal levels of available Zn for plants, especially at the root-soil interface (Norvell, 1972). More details will be discussed in the following section.

I.2.2. Zn availability to plants and factors affecting it

According to Le Claire *et al.* (1984), the water-soluble, exchangeable and organic pools of Zn are considered to be very bioavailable, the carbonates potentially available, and the oxides and residual fractions nonlabile or nonavailable. There is

disagreement over the best measurement for availability of Zn to plants. However, most researchers found Zn that is extractable by diethylenetrinitrilopentaacetic acid (DTPA) is often correlated with the available fractions of Zn in the soil (Le Claire *et al.*, 1984; Rappaport *et al.*, 1986; Shuman, 1986, 1988b) and Zn uptake by plants (Iyengar *et al.*, 1981). Thus, DTPA-extractable Zn is often considered to be the plant available fraction. The critical level for soil Zn depends on both soil and crop. Dr R. J. Hannam (unpublished data) suggested that, for wheat in most sandy soils in South Australia, it is likely to be at least 0.23 mg DTPA extractable Zn/kg soil.

The primary cause of low available Zn in soils is low Zn mineral content in their parent material and, for this reason, sandy soils, with quartzic parent materials low in Zn, are often reported Zn-deficient. Calcareous soils with high total Zn content are also frequently reported to be low in available Zn (Thorne, 1957; Navrot & Ravikovitch, 1969) as the solubility of Zn^{2+} in soils decreases as pH increases (Lindsay, 1972b) because of adsorption of Zn by carbonate (Leeper, 1952; Jurinak & Bauer, 1956). Leaching in high rainfall climates is also a major factor causing the low Zn in acid soils in which more of the Zn is in available forms (Lindsay, 1972b).

In submerged soils, such as flooded rice paddy fields, the soils' redox potential decreases and thus the availability of Zn increases because the occluded Fe and Al oxides dissolve (IRRI, 1970; Ponnamperuma, 1972). However, as the flooded conditions are prolonged and the redox potential continues to decrease, insoluble sulphides of Zn may be formed, decreasing the availability of Zn to crops (Ponnamperuma, 1972; IRRI, 1972; Harmsen & Vlek, 1985). The cycle of soil wetting and drying, in general, tends to decrease Zn in the soil solution and the weakly adsorbed fractions (Nambiar, 1975) due to adsorption and chelation with organic matter (Estepp & Keefer, 1969) or entrapment in the clay lattice of the expanding clay minerals (Reddy & Perkins 1974). Thus, Nambiar (1975) studied the effects of the top soil drying on Zn uptake by plant roots and showed that in his conditions it had only little effect on Zn uptake by plant roots.

Exposed subsoils have been reported to be Zn deficient (Viets, 1951; Grunes *et al.*, 1961), possibly because of the low organic matter or the high pH and carbonate in the subsoils (Lindsay, 1972b). Soils with restricted root zones due to hardpans, high water tables and compacted areas caused by tractor wheels were also found to be Zn deficient (Lucas & Knezek, 1972).

Soil organic ligands, produced either by root exudation or by micro-organisms during decomposition of the organic debris in soils (Stevenson & Ardakani, 1972), influenced availability of the rhizosphere-Zn in the soils and/or solutions with marginal levels of Zn available to the plant (Norvell, 1972). In the presence of organic ligands, Zn adsorption on clays and hydrous oxides has been shown either to increase or decrease and the effects on availability could be considered in two ways. Positively, the soluble organic anions may bind to the adsorbing surfaces and thus increase the negative charges for Zn adsorption (Parfitt & Russell, 1977; Barrow, 1985). In contrast, the humic and fluvic acid ligands from organic matter decomposition could form strong insoluble complexes with Zn which becomes less available. Alternatively, those ligands in soil solutions may compete with the adsorbed surfaces for Zn and hence decrease Zn adsorption, which has been suggested by Chairidchai & Ritchie (1990) to be the case in lateritic soils at low Zn concentration. It has been shown from their experiments that, besides pH and species of Zn ion in the solutions, adsorption was decreased most by citrate, followed by oxalate, tricarballylate, humate, salicylate and acetate; and none by catechol.

Low soil temperatures may restrict the release of Zn from organic matter by inhibiting both microbial activity and the establishment of the plant root system, and may therefore enhance Zn deficiency in temperate field crops (Lindsay, 1972b).

High levels of available phosphorus in soil were reported to induce Zn deficiency but the actual cause of this is still inconclusive (Boawn & Brown, 1968);

nitrogen has similar effects, though they are not as dramatic as those of phosphorus (Lindsay, 1972b).

Plants differ widely in their ability to obtain Zn from soil, not only among species but also among varieties (Lucas & Knezek, 1972). This aspect has been described in detail by Brown *et al.* (1972) and Graham *et al.* (1992).

I.3. GEOGRAPHIC DISTRIBUTION OF ZN DEFICIENCY

It is difficult to define the geographic pattern of Zn deficiency because of the influence of various environmental factors that reduce or enhance Zn availability to plants, and because there are differences between plant species and even among different varieties within a species in the efficiency of Zn uptake and use.

Ryan *et al.* (1967, cited by Lindsay, 1972b) reported low Zn levels in 10 of 15 European countries and in Israel. Sillanpää (1982) reported that there is a shortage of soil Zn throughout the world, especially in tropical and Mediterranean regions such as Italy, Syria, Lebanon, and Turkey; in Iraq, Nepal and Tanzania; in tropical Latin America such as Mexico and Brazil (Léon *et al.*, 1985). In Africa, lime-induced Zn deficiency has been reported in Nigeria, Guinea, the Ivory Coast, Sierra Leone, Sudan and Zimbabwe (Kang & Osiname, 1985). In Asia, Zn deficiency is found more frequently in the arid and semiarid regions such as India, Pakistan, Sri Lanka, northeastern Thailand, rice production areas in the Philippines (Katyal & Vlek, 1985), the north-central plains of West Java, the tropical soils of Taiwan, and the poorly drained, calcareous paddy soils of China (Zheng *et al.*, 1982 cited by Welch *et al.*, 1991). In the USA, there are reports from the Western regions where the soils are calcareous; the Southeast where the soils are sandy, well-drained and acid, or soils developed from phosphatic rock parent materials as in Kentucky and Tennessee; and the Florida citrus-producing Lakeland where the major soils are thermic, coated Typic Quartzipsamments and associated series (Welch *et al.*, 1991).

Most types of soils in the important cropping areas of Australia are severely Zn-deficient. Millikan (1938) reported Zn deficiency in the grey soils of the Wimmera district, and the Mallee region of Victoria. Donald & Prescott (1975) reported Zn-deficient soils such as the terra rossas, sandy podzolics, solidised solonetz, groundwater rendzinas and calcareous sands of the South-East of South Australia, the podzolic, lateritic podzolic, yellow earths and calcareous sands of the south-west of Western Australia, and the black earths of Queensland and New South Wales. Recently, Hannam (1990) reported Zn deficiency in the soils of the Murray Mallee and Eyre Peninsula in South Australia. Zn deficiency is always found in the acid sandy or gravelly lateritic soils formed over granitic gneiss, the southern coastal soils formed from sand on clay over Jurassic sediments. The western wheat belt, in the state of Western Australia, where the soils are grey and brown loams and sands formed from aeolian calcareous or leached siliceous sands over limestone, is considered to be the largest single area of Zn deficiency in the world (Welch *et al.*,1991).

I.4. ZN IN PLANTS

I.4.1. Uptake by plants

The form of Zn absorbed by plant roots has not been precisely defined. Generally, soluble forms of Zn are readily available to plants and there is agreement among reports on the predominant uptake of both hydrated Zn and Zn^{2+} (Kabata-Pendias & Pendias, 1984; Halvorsen & Lindsay, 1977). However, there is evidence suggesting several other complex ions and Zn-organic chelates may also be absorbed (Loneragan, 1975; Weinberg, 1977, cited by Kabata-Pendias & Pendias, 1984).

As well as the forms of Zn taken up, there is still controversy over whether the uptake process is active or passive. The uptake of Zn has been reported to be linear with concentration in nutrient solution and in soils; the absorption rate differs greatly among plant species, and depends on growth media and composition of the nutrient solution, the

presence of Ca being particularly important (Kabata-Pendias & Pendias, 1984). There are a number of early research reports (for example, Moore 1972, Rathore *et al.*, 1970) supporting a passive absorption process. It is considered that root cells often maintain potential across the plasma membrane in the range of -120 to -180 mV. According to the Nernst equation, if a divalent cation is passively distributed across the plasma membrane, a membrane potential of -120 mV could balance an accumulation ratio of 10,000:1. Therefore, the transport process of Zn in an unchelated form into root cells could be a passive ion flux through divalent cation channels (Kochian, 1991). It should be noted that describing this kind of absorption mechanism as passive ignores the energy required to maintain the transmembrane potential difference.

However, Schmid *et al.* (1965) observed, in barley roots, a steady rate of $^{65}Zn^{2+}$ uptake for the first 2 h which was markedly reduced by low temperature and metabolic inhibitors. It is also reported from this work that 60% of the Zn was taken up by diffusion and bound in the apoplasm, and the rest was due to a plasma membrane transport-mediated process. More recently, Santa Maria & Cogliatti (1988), using compartmental analysis techniques, reported the efflux of Zn^{2+} across the membrane to be 65 to 80% of the influx; three subcellular compartments were assigned according to their half-time ($t_{1/2}$) for exchange of Zn^{2+}: 8 to 14% of the total root content was assigned to the Donnan free space with a $t_{1/2}$ of 0.08 h, the cytoplasm was assigned about 8% root content with a $t_{1/2}$ of 0.55 h and the remaining 76% was the slowest exchanging compartment with $t_{1/2}$ of 134 h and believed to be more complicated than the simple vacuolar Zn. More research is certainly needed for better understanding in this aspect.

I.4.2. Location and forms of Zn within plants

Once in the plant cell, Zn, as well as the other micronutrient cations, is bound as low-molecular-weight organic complexes so that the activity of the free ion is maintained at a low enough level to be non-toxic to the cell (Hendry & Brocklebank, 1985). The nonprotein amino acid, nicotinamide, was speculated to play an important role in the

symplastic transport of most micronutrient cations including Zn (Rudolph & Scholz, 1972 cited by Kochian, 1991; Budesinsky *et al.*, 1980; Scholz *et al*, 1985, 1987). The formation of Zn-phytate and other insoluble Zn complexes was also reported by Weinberg (1977 cited by Kabata-Pendias & Pendias, 1984) and Tinker (1981). More recently, Grill *et al.* (1985, 1987) discovered a class of heavy-metal-binding peptides called phytochelatins which are a chain of two to eight repeating glutamine-cysteine residues with a glycine residue at the carboxy-terminus. Their molecular weight ranges from 1000 to 1500 in castor bean (Van Goor & Wiersma, 1967), up to 4000 in orange and grapefruit trees (Taylor *et al.*, 1988). These compounds have been hypothesized not only to be responsible for the detoxification of the heavy metal in plants, but also to be involved in the inter- and intra-cellular movement of Zn^{2+}, Cu^{2+} and Ni^{2+} by chelating these cations as they enter the plant cells (Grill *et al.*, 1987; Robinson & Jackson, 1986; Walker & Welch, 1987).

Zn is likely to be concentrated in mature leaves (Kabata-Pendias & Pendias, 1984). However, Scheffer *et al.*(1979 cited by Kabata-Pendias & Pendias, 1984) reported the highest Zn content of barley leaves, sheaths, and internodes to always be during the phase of intensive growth, which embodies a large flux of Zn within the plant during the vegetative period. Yläranta *et al.* (1979) on the other hand, reported that variation in the Zn content of wheat is surprisingly small and that the content increases slightly throughout the whole growth period. It has been calculated by Baumeister and Ernst (1978, cited by Kabata-Pendias & Pendias, 1984) that up to 75% of the total Zn that is taken up is in the tops of young plants, whereas only 20 to 30 % occurs in the tops of old plants. Zn is reported to be accumulated in seeds with the highest concentration in the embryo (Riga & Bukovac, 1961 cited by Shkol´nik, 1984). This is in agreement with Milthorpe & Moorby's (1979) suggestion that Zn is one of the soil minerals needed by seedlings during emergence to achieve maximum growth rate. A high level of Zn was also detected in the protoplasm of the oosphere and synergids of the female gametophyte cells (Fursov, 1965 by Shkol´nik, 1984). In addition to this, abnormal pollen grain development was reported in Zn-deficient wheat (Sharma *et al,* 1979) and maize (Sharma

et al., 1987). This stresses the important role of Zn in plant reproductive processes.

Roots often contain much more Zn than do tops, particularly if the plants are grown in Zn-rich soils. With luxury levels of soil Zn, this element may be translocated from the roots and accumulated in aerial parts of the plants. Zn is reported to be concentrated in chloroplasts, especially in those of some plants (e.g., spinach). This metal is also likely to be accumulated in vacuolar fluids and in cell membranes (Tinker, 1981).

I.4.3. Translocation

Very little research has been conducted on the translocation of Zn in plants. Zn has been reported in xylem exudate of various plants ranging from 0.3 to 1.4 mg Zn/l (Clark *et al.*, 1986; Hocking, 1980; Hocking *et al.*, 1978; White *et al.*, 1981b). Tiffin (1972) tried to investigate forms of Zn in the xylem but was unclear whether the form was Zn^{2+} ions or complexes of organic acid. However, White *et al.* (1981a) suggest that Zn forms complexes with citric and malic acids in soybean and tomato xylem. Fractions of Zn bound to light organic compounds in xylem fluids and in other plant tissue extracts may suggest its high mobility in the plant (Van Goor & Wiersma, 1976; Tinker, 1981).

Hocking (1980) found, in *Nicotiana* spp., that levels of Zn in phloem were 10 times higher than in the xylem of the same plant. The forms of Zn in phloem are reported to be anionic organic complexes of phytochelatins as previously mentioned in section I.3.2. According to results from experiments in wheat using split root systems, half with and half without Zn in the root medium, Loneragan *et al.* (1987) suggested that Zn could be translocated in the plants' phloem tissue with a rate up to 0.7 µg Zn/g dry root/day and Zn-adequate wheat plants could translocate sufficient Zn in their phloem to maintain growth of that part of their root system not supplied with Zn. When luxury levels of Zn are supplied, several plant species have mobilized appreciable quantities of this metal from old leaves to generative organs, but under Zn-deficiency conditions the same

species have mobilized little, if any, Zn from old leaves (Kabata-Pendias & Pendias, 1984).

I.4.4. Interactions between Zn and other elements

Several anions such as HPO_4^{2-} and $H_2PO_4^-$, NO_3^-, and SO_4^{2-} have been shown to inhibit Zn uptake and absorption (Rashid *et al.*, 1976; Ozanne, 1955; Leece, 1978; Panavasasivam & Axley, 1982). Most of the alkali cations - NH_4^+, Rb^+, K^+, Ca^{2+}, Na^+,Mg^{2+}, Ba^{2+}, Sr^{2+} and Li^+ have been shown to inhibit Zn absorption (Chaudhry & Loneragan, 1972a). Other cations known to inhibit Zn absorption are Cu^{2+} and H^+ (Chaudhry & Loneragan, 1972b), Mn^{2+} and Fe^{3+} (Rashid *et al.*, 1976).

Concentrations of many elements have been shown to increase in Zn deficient plants, for example : Fe, Mn, Ca, Mg, Cu and P (Chaudhry *et al.*, 1977; Ishizaka, 1971; Loneragan, 1951; Loneragan *et al.*, 1979; O'Leary, 1971; Rovira, 1965; Tomiyama *et al.*, 1983). Graham *et al.* (1987) showed the accumulation of B, P, NO_3^-, S, Ca, Mg, K and Cu in Zn deficient barley plants grown in nutrient solutions. This phenomenon was explained by the role of Zn in regulating ion transport across the cell membrane. For P, Webb (1987) illustrated, by an experiment using radioactive phosphate, that Zn deficiency induced P toxicity by enhancing P accumulation in shoots, as a result of enhanced gross transport of P to shoots and depressed export of P out of these old leaves.

I.4.5. Symptoms of Zn deficiency & critical concentration

Visual symptoms of Zn deficiency vary among plant species (Rahimi & Bussler, 1978; Cakmak & Marschner 1988b). In wheat, Snowball & Robson (1983) stated the first visible symptom of Zn deficiency to be a colour change from the normal green to a grey-green towards the middle of the middle-aged leaf; then necrotic lesions are developed in these areas and they gradually extend towards the leaf margins (Plate 1). As the situation becomes more severe, the leaves often look 'oily' and the areas around the

necrotic lesions become a mottled yellow-green. At this stage the leaves often collapse in the mid regions and symptoms develop on even the youngest leaves. Sharma *et al.* (1979) reported, in older wheat plants, that Zn deficiency could delay heading and anthesis and cause abnormal development of pollen grains.

Levels of Zn in plants range from 10 to 100 mg/kg for most crops and pasture plants (Chapman, 1966; Gladstones & Loneragan, 1967). Although the critical Zn concentrations seem to differ between plant species and environmental conditions, it has been found that 1-3 mg soluble Zn/kg dw was sufficient for most of the plant species (Rahimi & Schropp, 1984; Cakmak & Marschner, 1988b). A critical concentration range for cereals, measured as total Zn concentration in the youngest expanded leaf blade (YEB), has been reported to be 16-25 mg Zn/kg dry weight, with the average of 20 mg Zn/kg dry weight (Reuter & Robinson, 1986).

I.5. ROLES OF ZN IN PLANTS

The role of Zn as a constituent and activator of numerous enzymes has made it important for both plant and animal metabolism. Sixty enzymes representing almost all enzyme groups have been found to be Zn metalloenzymes (Riordan, 1976). Therefore, the metabolic functions of Zn are various, involving carbohydrate, protein and lipids, as well as metabolism of growth factors, cell division, growth and development of plants (Shkol´nik, 1984; Marschner, 1989).

I.5.1. Detoxification of the activated oxygen species

A major activated oxygen species is the superoxide radical, $O_2^{\cdot -}$, formed in living cells during respiration and photosynthesis (Elstner, 1982), at various locations in the cytosol (Kellogg & Fridovich, 1975), cell wall (Halliwell, 1978; Mader & Amberg-Fisher, 1982), mitochondria (Takeshige & Minakami, 1979) and chloroplasts (McRae & Thompson, 1983; McRae *et al.*, 1985). $O_2^{\cdot -}$ can form other species of more reactive radicals such as hydrogen peroxide (H_2O_2), hydroxyl radical ($OH\cdot$) and also singlet

Plate 1 Zn deficiency symptoms in a wheat plant as necrotic spots in the middle region of the youngest expanded blade.

oxygen ($^1O_2$) which, while not a radical, is very reactive. It has been suggested that the highly reactive OH· firstly causes lipid peroxidation, then alkoxy radicals (RO·) and peroxy radicals (ROO·) are formed which initiate another chain reaction of lipid peroxidation (Elstner, 1982), destabilising the membrane bilayer structure and increasing membrane fluidity (Thompson *et al.*, 1987). Free radicals are also able to inactivate proteins (Wolff *et al.*, 1986), predispose them to proteolysis by changing their conformation or increasing the susceptibility to proteolysis by oxidation, and even fragment them (Thompson *et al.*, 1987). Elstner (1982) reported the damage of polysaccharides and nucleic acids by those active free radicals.

The enzyme, superoxide dismutase (SOD), which converts $O_2^{\cdot -}$ into H_2O_2, and catalase which converts H_2O_2 into H_2O (Cakmak & Marschner, 1988c) is the mechanism that living cells use to prevent an excessive accumulation of $O_2^{\cdot -}$ (McCord & Fridovich, 1969). There are two families of three dismutases. The first family is iron dismutase found mostly in the prokaryotes, and manganese dismutase found in both prokaryotes and eukaryotes. The other is copper-zinc dismutases (Cu-Zn SOD), dimers with native molecular weight around 31,000 to 33,000, containing nearly 2 g-atom of Cu and 2 g-atom of Zn per mole, found only in higher eukaryotic fungi, plants and animals (Steinman, 1982). In plants, Cu-Zn SOD enzyme is present in the chloroplasts of the green tissue, in the cytosol of the nongreen tissue, and a small amount in the space between the inner and the outer mitochondrial membrane (Halliwell, 1982). The role of Zn in this enzyme has not been fully understood. However, evidence up to now suggests the importance of Zn in stabilizing its active site (Steinman, 1982); the concurrence of low SOD activity and high levels of $O_2^{\cdot -}$ have been reported in Zn-deficient plants by Vaughan *et al.* (1982); Cakmak & Marschner (1988a; c).

I.5.2. Protein metabolism

In Zn-deficient plants, RNA content, rate of protein synthesis and protein content are noticeably reduced (Kessler & Monselise, 1959); parallel to this, the

accumulation of non-protein, soluble nitrogen compounds, free-amino acids and amides, occurring specifically under Zn deficiency, are increased (Steinberg, 1956, cited by Shkol´nok, 1984; Possingham, 1957; Naik & Asana, 1961). This demonstrates that Zn deficiency can inhibit protein synthesis in plants.

Marschner (1989) suggested that there are at least three mechanisms involved. Firstly, Zn may have a role as a structural component of the enzyme RNA polymerase since there are about two atoms of Zn per molecule of RNA polymerase I, and the enzyme is not active if those Zn atoms are removed as a result of Zn deficiency (Falchuk *et al.*, 1977; Soloiman & Wu, 1985). Secondly, the reduction of Zn content in ribosomal RNA, from 650-1280 μg Zn/g RNA in Zn-sufficient *Euglena* cells to 300-380 in deficient ones (Prask & Plocke, 1971) may cause ribosome disintegration as Zn is also a constituent responsible for ribosome structural integrity (Obata & Umebayashi, 1988). Lastly, the rate of RNA degradation may be accelerated (Cakmak *et al.*, 1989) through increasing activity of RNase enhanced by Zn deficiency (Sharma *et al.*, 1982), as RNase activity has been shown to correlate inversely with both Zn supply and protein content (Simon, 1979).

In addition to this, some evidence suggests the importance of Zn in transcription. For example, many nucleotidyltransferases proved to contain Zn (Valenzuella *et al.*, 1973) and Zn is identified as an integral part of RNA-dependent DNA-polymerase or reverse transcriptase (Springate *et al.*, 1973). More recently, intrinsic Zn atoms have been found in the DNA-binding regions of the transcription factors of eukaryotes and their transcription activity is dependent on the presence of these Zn atoms (Brandon & Tooze, 1991).

I.5.3. Carbohydrate metabolism

Zn may affect carbohydrate metabolism by impacting on the carbohydrate synthesis in plants or on photosynthesis, and on the carbohydrate utilization or respiration. In many Zn-deficient plants, the concurrence of reducing sugar accumulation

and sucrose and starch reduction has been reported, as well as the accumulation of organic acids, which has been suggested as an indication of impaired sugar phosphorylation processes (Shkol´nik, 1984).

I.5.3.1. Effect of Zn on photosynthesis

There are number of reports, cited by Shkol´nik (1984), of direct and indirect involvement of Zn in photosynthesis even though the role of Zn in the process itself has not been confirmed. The direct effects of Zn deficiency in promoting abnormal cellular development in the plant leaves, particularly of chloroplasts, have been reported (Reed, 1938; Vesk *et al.*, 1966; Shkol´nik, 1984). Vesk *et al.* (1966) measured Zn concentration in chloroplasts and proposed a role for Zn in maintaining their structure, and Sharma *et al.* (1982) suggested that the disturbed chloroplast structure under extreme Zn deficiency may inhibit net photosynthesis. Singh (1988) reported that application of Zn at up to 5 mg/l increased the chlorophyll concentration in seedlings of *Indigofera glandulosa* grown in pot culture. Glutamate dehydrogenase in tomato chloroplasts is also a Zn metalloenzyme (Igoshina & Kositsyn, 1975 cited by Shkol´nik, 1984). Zn was also reported involving in the biosynthesis of some chlorophyll precursors such as protoporphyrin (Shuvalov & Kranovsky, 1971 cited by Shkol´nik, 1984) and IX porphobilinogen (Komai & Neilands, 1968 cited by Shkol´nik, 1984).

One indirect effect of Zn on photosynthesis might be through the Zn metalloenzyme carbonic anhydrase (CA). Carbonic anhydrase, which facilitates the diffusion of CO_2 used in photosynthesis through both plasmamembrane and tonoplast by catalyzing the reversible hydration of CO_2 (Waygood *et al.*, 1969; Hatch & Slack, 1970), was the first enzyme found to contain Zn (Tobin, 1970; Risiel & Graff, 1972). In Zn deficiency, the reduction in activities of ribulose bisphosphate carboxylase oxygenase in C_3 plants (Jyung *et al.*, 1972) and phosphoenol pyruvate carboxylase in C_4 plants (Shrotri *et al.*, 1983), both of which receive CO_2 as their substrate from CA, might be considered to be a result of a decline in activity of CA. Randall & Bouma (1973), however, found that although CA activity significantly decreased in the Zn-deficient

spinach leaf, only the very lowest CA activity could reduce photosynthetic rate and, thus, concluded that there was no close relationship between photosynthesis and CA activity.

In conclusion, although the rate of photosynthesis has been reported to decrease under Zn deficiency (Fujiwara & Tsutsumi, 1962 cited by Shkol´nik, 1984), the critical role of Zn in this process is still unclear.

I.5.3.2. Effect of Zn on respiration

It was indicated from the experiments of Paribok (1972 cited by Shkol´nik, 1984) that Zn deficiency strongly inhibited glycolysis, the Krebs cycle and the respiratory electron transport chain, which are the three major pathways of respiration. A considerable amount of Zn was also found in the mitochondria (Kathore *et al.*, 1972) which were found to be fewer (Price & Brown, 1965) and abnormally formed in Zn-deficient conditions (Reed, 1938). Moreover, Zakharchishina & Klyuchko (1970, cited by Shkol´nik, 1984) showed a decrease in the energy efficiency of mitochondrial respiration in barley plants with inadequate Zn supply.

In anaerobic conditions, the Zn-containing enzyme alcohol dehydrogenase (ADH) plays an important role in anaerobic root respiration (eg. in the root apex of flooded rice), catalyzing the reduction of acetaldehyde to ethanol (Marschner, 1989). The activity of ADH is markedly reduced with Zn deficiency, thus impairing the anaerobic root metabolism in such conditions (Moore & Patrick, 1989).

I.5.3.3. Effect of Zn on carbohydrate partitioning

According to Marschner (1989), the activity of fructose 1,6-biphosphatase which is a key enzyme in the partitioning of C_6 sugars in the chloroplasts and the cytoplasm, has been shown to decline rapidly under conditions of Zn deficiency.

Aldolase isoenzymes, which regulate the C_3 photosynthate transfer to the cytoplasm when present in the chloroplast and the C_6 sugar transfer to the glycolytic

pathway when present in the cytoplasm, are another group of key enzymes affected by Zn deficiency. O'Sullivan (1971) reported the severe and specific reduction in aldolase activity of various Zn-deficient plant species and suggested this as an indicator of the Zn nutritional status in plants. However, Bar-Akiva *et al.* (1971) showed a reduction in aldolase was not observed in citrus leaves. Finally, as a constituent of phosphoglycerate aldehyde dehydrogenase, Zn may be involved in the reductive transformation of photosynthetic products (Shkol´nik, 1984).

The concentrations of carbohydrates in leaves of Zn-deficient plants are, however, either unaffected or increased (Marschner & Cakmak, 1989) and excretion of sugars at the leaf surface can be observed with severe Zn deficiency (Marschner, 1989). Furthermore, Zn deficiency has been shown to depress starch concentration and soluble starch synthetase activity in bean plants (Jyung *et al.*, 1975) but to enhance starch and sugar concentrations in cabbage (Sharma *et al.*, 1982). Thus, Marschner (1986) speculated that changes in carbohydrate metabolism induced by Zn-deficiency might not be primarily responsible for either growth retardation or visible deficiency symptoms.

I.5.4. Membrane integrity & permeability

The direct association of Zn with cell membranes has been well studied in animals in which Zn was found to influence the energy-dependent permeability of mitochondrial membranes to potassium and magnesium (Brierley & Knight, 1967; Brierley & Settlemire, 1967). This function of Zn was postulated to be due to the binding of Zn to sulphydryl groups of membrane proteins (Chvapil, 1973) and/or phospholipids (Von Glos & Bournell, 1981).

In plants, the initial evidence was based mostly on comparing the balance of external and internal ions between Zn deficient and Zn adequate plants, since Epstein (1961) showed that Zn and Ca might be concerned with regulating ion transport across the cell membrane. The first direct implication of a Zn effect on the integrity of plant cell membranes, based on this view, was that of Welch *et al.* (1982) in their study of Zn

deficiency-induced P toxicity. The inconsistency between the higher P content and the lower short-term P absorption rates in the Zn-deficient roots compared to the Zn adequate roots led to the conclusion that Zn-deficient roots were leaking greater quantities of P than the Zn-adequate roots during the desorption period. The permeability of the root membranes can also be measured as the loss of Rb or K, or the net leakage of soluble amino acids and reducing sugars from the root system (Ratnayake *et al.*, 1978).

Root exudates, being compounds of low molecular weight leaking from root cells non metabolically mediated (Rovira,1979), were used as an indicator for root plasma membrane permeability. Cakmak & Marschner (1988a) measured root exudates in cotton, wheat, tomato and apple by incubating roots of intact plants in aerated $CaCl_2$ (0.5mM) solution. In all plants studied, Zn deficiency increased root exudation and reapplication of Zn to Zn deficient plants not only increased Zn concentration in the plant, but also decreased root exudation. Omission of Ca from the incubation solution increased root exudation by a factor of about two in the Zn sufficient plants but only slightly in the Zn deficient plants.

The effects of Zn^{2+} on plasma membrane permeability which are linked to superoxide radical formation and peroxidation, were also studied in cotton roots (Cakmak & Marschner, 1988b). In Zn deficient plants, the plasma membrane permeability was increased, as indicated by a 3-, 5-, and 2.5 fold increase in root cell leakage of K^+, $NO3^+$ and organic carbon compounds, respectively, compared to the Zn sufficient plants; resupply of Zn to the deficient plants substantially decreased this leakage. The levels of $O_2^{\cdot -}$ and the NADPH-oxidase activity were also higher in the Zn deficient plants than in the sufficient ones, and resupply of Zn to the Zn deficient plants decreased these two measurements. Because the NADPH-derived free radicals are involved in the peroxidation of unsaturated fatty acids in microsomal, mitochondrial, nuclear and plasmalemma membranes, these results suggest that Zn^{2+} directly affects integrity of the plasma membrane by inhibiting $O_2^{\cdot -}$ generated by a membrane-bound NADPH oxidase. The same results were also confirmed in bean and tomato, in parallel with the reduction in

SOD activities and catalase in the Zn deficient plants. This leads to the conclusion that Zn deficiency reduces membrane integrity and/or increases membrane permeability by enhancing $O_2^{\cdot -}$ production and impairing $O_2^{\cdot -}$ detoxification due to the reduction in SOD activity, which in turn increases membrane peroxidation (Cakmak & Marschner, 1988c).

Zhang *et al.* (1989) found that, in Zn deficient wheat and barley root exudates, the same compounds which could mobilise Zn from either a selective cation exchanger (Zn chelite) or a calcareous soil, could also mobilize Fe under Fe deficiency. When visual Zn deficiency symptoms in the leaves had appeared these compounds were exuded much more from Zn deficient than Zn sufficient plants, and the release followed a distinct diurnal pattern with the maximum between 2 and 8 h after the onset of light. The mobilizing compounds released under both Zn and Fe deficiency, known as phytosiderophores, were identified to be dominantly 2'-deoxymugeneic acid in wheat and epi-3-hydroxymugeneic acid in barley.

Since the 'leakage' took place even when the Zn concentration inside the root was considered to be sufficient for plant growth (Better & O´Dell, 1981; Welch *et al.*, 1982; Loneragan *et al.*, 1986), it was suggested that the role of Zn in the root cell membranes may be due to Zn deficiency external to the membrane surrounding the cell rather than deficiency in intracellular Zn. This implies that membrane leakage might happen as soon as Zn concentration in the rhizosphere drops to the deficiency level while the plant itself still has sufficient Zn within its cells.

I.5.5. Metabolism of growth hormone

One of the symptoms of Zn deficiency is growth retardation, especially of the internode, which is closely associated with the reduction of auxin concentration in Zn deficient plants (Shklo´nik, 1984). Skoog (1940) found both free and bound auxin concentrations were reduced, and resupplying Zn caused an increase in those concentrations and growth resumed within 24 hours. Tsui (1948) observed that, in Zn-deficient tomato, the reduction of auxin concentration occurred before any detectable

growth reduction or visual deficiency symptoms appeared.

There are reports of a decrease in tryptophan, a precursor of IAA, in Zn deficient fungus *Neurospora* (Nason, 1950) and in higher plants (Tsui & Wu, 1960); an increase in tryptophan content with increasing Zn application was reported in rice grain by Singh (1981). A decrease in auxins, or indolacetic acid (IAA), under condition of Zn deficiency is speculated to be through the synthesis of its precursor, tryptophan (Shklo´nik, 1984, Marschner, 1989) . One possible pathway of tryptophan synthesis is from indole and serine, and Zn deficiency was suspected to inhibit serine synthesis (Shklo´nik, 1984). Alternatively, Takaki & Kusushizaki (1970) suggested that Zn deficiency might interrupt tryptophan synthesis through the conversion of tryptamine to IAA as they found levels of both tryptophan and tryptamine were higher in the Zn deficient plant than the normal. To support this idea, Suge *et al.*(1986) showed, in Zn deficient maize, a lower activity of gibberellin-like substances, which are thought to promote the conversion of tryptophan and tryptamine to IAA. The suppression of tryptophan under conditions of Zn deficiency may also be due to decreasing levels of vitamins B1 and B6 which are known to have a major role in combining ammonium with organic acids in tryptophan synthesis in tomato (Shkol´nik, 1984). Another possible role of Zn in IAA synthesis may be through regulating the activity of aminotransferase which is the key enzyme in the biosynthesis of the indole and phenol types of growth regulators (Rudakova *et al.*, 1981, cited by Shkol´nik, 1984).

Because of differences in opinion about IAA synthesis in different plants, this effect of Zn deficiency still needs investigation.

PART II : *RHIZOCTONIA* ROOT ROT IN CEREALS

II.1. IMPORTANCE

Rhizoctonia solani Kühn was first identified more than 100 years ago by Kühn (1858). It occurs world-wide, being a destructive and versatile plant pathogen, attacking

an extensive range of plants, causing seed decay, damping off, stem cankers, root rots, fruit decay and foliage diseases. The study of its life cycle and control has been the subject of extensive research by numerous investigators (Parmeter, 1970). *Rhizoctonia* root rot of cereals was first reported by Samuel (1928) as bare patch disease in South Australia, and research on this fungus at the Waite Institute was initiated then.

II.2. DISTRIBUTION

Besides Australia, *Rhizoctonia* root rot of cereals, is known in Scotland as 'barley stunt disorder' (Dillon-Weston & Garret, 1943), in Canada (Benedict & Mountain, 1956) as bare patch disease and it has been reported as 'crater disease of cereals' in South Africa (Scott *et al.*, 1979). More recently, the disease has been reported in the Pacific Northwest of USA in Washington and Idaho (Weller *et al.*, 1986), Oregon (Pumphrey *et al.*, 1987), and Georgia (Rothrock & Hargrove, 1987). Pumphrey *et al.*(1987) reported 50% reduction in straw and grain of infected plants because of the reduction in panicle numbers.

II.3. DISEASE SYMPTOMS

Cereal plants attacked by *Rhizoctonia* root rot disease show symptoms both above and below ground (Plates 2 & 3). As described by Samuel (1928) and Samuel & Garrett (1932), severely diseased plants are noticeable at a very early stage, frequently before the crop has begun to tiller. The above-ground symptom is uneven growth in infected fields, with well defined patches, varying from a few meters in diameter to very large areas, which become more noticeable as the crop grows up owing to the hollow space left. In some cases the patches have persisted in the same spots in the field over several years and have even enlarged in spite of a year's fallow. The affected plants do not tiller and usually remain practically at a standstill during the winter months of June, July, and August. They appear spindly and stunted, with stiff, rolled leaves pointing upwards, and sometimes look purple. Then, as the weather becomes warmer, all the plants in many of the patches die at a height not more than about 15 cm. When the attack

is not so severe, some of the plants are able to form fresh roots and may make a fairly good recovery, but may be delayed in maturity.

Roots of the diseased plants, when washed out, show unique symptoms. It is found that the tips or intermediate portions of the root are so vigorously attacked that the tissue loses all turgidity. The flaccid, water-soaked appearance of the tissues in this earliest stage does not last long before the roots die back from the tips and turn brown. The cortex also rots away (or 'cortical truncation') and the central cylinder or stele breaks, so the effect is of a brown stub or 'spear tip'. The killing of root tips and other parts of the roots may stimulate the formation of laterals, which are in turn attacked. The result is a much-branched root system in diseased plants, mainly in the seminal roots, for the roots initiated from the first node are usually quickly killed back. This is a severe effect of the fungus on the plants, for they are unable to establish any secondary root system and therefore die.

The causal organism seen and isolated from the flaccid portions of root in the earliest stages of infection has been proved to be *Rhizoctonia solani* (Samuel & Garrett, 1932; Kerr, 1955; Flentje, 1956; de Beer, 1965; Neate & Warcup, 1985). This stage is transitory and by the time the root has progressively rotted back to a stump, it is almost impossible to find any active-looking hyphae, and this may explain why it is very difficult to isolate the fungus from the infected roots at later stages.

II.4. CHARACTERISTICS OF THE FUNGUS

The genus *Rhizoctonia*, a group of basidiomycetous imperfect fungi, was established in 1815 by De Candolle, and more than one hundred species have been claimed to belong to this genus (Parmeter *et al.*, 1970). However, only forty-nine have been confirmed to be true *Rhizoctonia* spp. (Ogoshi, 1987). *Rhizoctonia solani* Kühn is the multinucleate imperfect state of *Thanatephorus cucumeris* (Frank) Donk (Frank, 1863; Talbot, 1970). The morphological characteristics described by Kühn (1858) and modified by Duggar (1915), Parmeter & Whitney (1970) and Ogoshi (1975), are:

Plate 2 Above ground symptoms of a wheat field infested by *Rhizoctonia* root rot. Note well-defined patch areas with stunted plants.

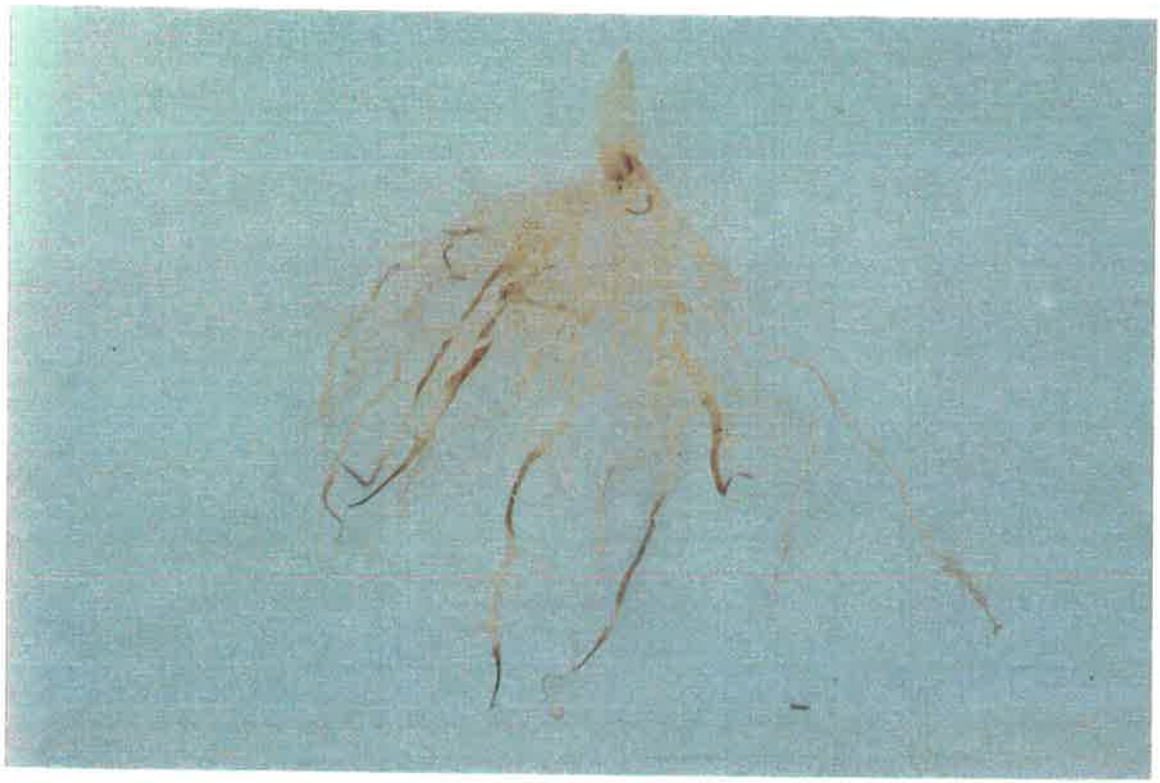


Plate 3 A root system of an infected plant from a bare patch area. Note the 'spear tip' and 'cortical truncation'.

a) branching near the distal septum of cells in young vegetative hyphae, b) constriction of hyphae and formation of septae a short distance from the point of origin of hyphal branches, c) the presence of dolipore septae, and d) the absence of clamp connections, conidia, rhizomorphs or sclerotia differentiated into rind and medulla.

The group causing bare patch or root rot disease in cereals was initially defined, because the symptoms appear only on the roots of the infected plants (Samuel & Garrett, 1932), as "root strains" (Kerr, 1955; Flentje, 1956 & De Beer,1965). Using the concept of anastomosis groups based on hyphal fusion between the two isolates, Neate & Warcup (1985) identified this strain to be anastomosis group 8 (AG-8). More recently, by using electrophoretic examination of the extracellular pectic enzymes or pectic zymogram produced by different isolates, Sweetingham *et al.* (1986) identified the isolates causing bare patches in cereals and lupins to belong to zymogram groups 1 and 2 (ZG_1&ZG_2). Neate *et al.* (1988) compared anastomosis to zymogram groups and found that isolates in AG-8 fell into ZG_{1-1}, ZG_{1-2}, ZG_{1-3} and ZG_2. Matthew (1991) used both polyclonal and monoclonal antibodies and DNA probes to identify various anastomosis groups of *R. solani* and found that AG-8 isolates were biochemically different from isolates in the other anastomosis groups found in South Australia.

II.5. GROWTH & SURVIVAL IN SOILS

Anastomosis groups of *R. solani* differ in their modes of survival, which are reported as sclerotia, short thick-wall hyphae (monilioid cells) or ordinary hyphae (Papavizas, 1970; Naiki, 1985; Neate, 1987). However, they hold in common the saprophytic characters of dormancy, especially the ability to survive in soils without any host for many years, even during very long dry periods (Boosalis & Scharen, 1959; Baker, 1970; Henis & Ben-Yephet, 1970; Papavizas, 1970; Baker & Cook, 1974).

According to Neate (1987), *R. solani* AG-8 was successfully isolated from hyphae, chains and bundles of monilioid cells, both in the soil and in the plant debris in the field, but no sclerotium or perfect state was found. It was isolated more frequently

from plant debris particles at 0-5 cm depth than at 5-10 cm or 10-15 cm depths. The particle size range from which the fungus was most frequently isolated was 750 µm to 2 mm. The most important sources of natural inoculum were the shoot and root debris particles from graminaceous plants, but the fungus could also be isolated from the residues of *Emex* spp., *Medicago* spp. and unidentified organic matter.

II.6. INFECTION

Kommedahl & Windel (1979) classified root diseases into two groups according to the fungus-root interactions. The first group is the host-dominant diseases in which the infection by the pathogens, mostly specialized, is controlled by resistance of the hosts. The other group, to which *R. solani* belongs, is the pathogen-dominant diseases, in which the pathogens, mostly unspecialized, actively control its infection and subsequent progression in the early growth stages of the host plant growth when the host shows little or no resistance to the fungus. At this stage of infection, the fungus is usually tissue-nonspecific, pathogenic races are uncommon and the immature plant tissues are attacked by enzymes, toxins, or both. In this group, *R. solani* was classified as a macerative pathogen which attacked the host plants by enzymes produced by the fungus.

It has been reported, in other groups, that penetration into the host tissue may occur either through the intact plant surface, natural openings or wounds (Dodman & Flentje, 1970) or the point of lateral root development (Kernkamp *et al.*, 1952; Wyllie, 1959). However, no information exists for AG-8 isolates. Several investigators have reported the formation of 'infection cushions', or aggregates of compacted hyphae which closely contact the surface of the host roots at the point of infection (Flentje *et al.*, 1963; Martison, 1965; Dodman & Flentje, 1970). However, Flentje *et al.* (1963), using the cellophane bag technique in which the seedlings were put in a sterile cellophane bag before planting into the soil incubated with the fungus, showed that symptoms could be developed on the seedling root without physical contact between seedling and the fungus mycelium in the surrounding substrate, and thus they concluded that the pathogenic reactions must have been due to diffusible materials. Kommedahl & Windels (1979)

suggested those materials to be the cell wall degrading enzymes, polygalacturonases and pectin lyase (Bateman & Miller, 1966; Bateman, 1967 & Lai *et al.*, 1968), rather than toxic substances. Cooper (1984) showed that endo-polygalacturonase, one of the pectic enzymes, is important for cell wall degradation of host plants by necrotic pathogens.

Although a number of works has shown seed and root exudates to be the important factors for the formation of infection structures, the role of individual components of exudates has not been determined (Kerr & Flentje, 1957; Flentje *et al.*, 1963 & Martinson, 1965). Martinson & Baker (1962) showed that growth of the fungus in soils could be stimulated by exudates from the host. The amount of exudate regulated the number of infection cushions (Flentje *et al.*,1964) and the amount of disease (Tousson & Patrick, 1963; Schroth & Cook, 1964). Exudate may be increased by restricted aeration (Woodcock, 1962), by the presence of phytotoxic decomposition products from organic matter (Toussoun & Patrick, 1963) or as the result of deficiencies in some nutrients, such as Zn deficiency as described in Section I.5.4.

II.7. CONDITIONS FAVOURING THE DISEASE

Tillage systems resulting in reduced soil disturbance are reported to be the most important factor increasing *Rhizoctonia* bare patch in the field. The disease was reported to be more severe in direct drilled wheat than in wheat sown into cultivated soil (Neate, 1984; Rovira, 1986; Pumphrey *et al.*, 1987; Smith & Wehner, 1988). Weller *et al.* (1986) reported that at all sites where the disease occurred, the wheat or barley was either direct-drilled (no-tillage) into stubble, sown with minimal prior tillage, or sown the same day the soil was tilled. In experimental plots with winter wheat, the number of patches per treatment was highest in the no-tillage, plots followed by reduced tillage and was least in conventional tillage plots. Smith & Wehner (1988) also reported greater disease severity in cereals grown in soil with a reduced tillage system in South Africa. Neate (1984) suggested that the reasons for increasing severity in the direct-drilling might be increasing plant debris which was the important source of inoculum in such tillage systems. More debris infected by the fungus was found in the soil from the direct-drilled

plots compared to the cultivated plots. The effects of the herbicide use and higher soil bulk density in direct-drilling were found not to be important in this study. However, Rovira & McDonald (1986), investigating the interaction between *Rhizoctonia* root rot severity and applied herbicides, reported an increase in disease severity and a yield reduction in barley crops grown after chlorsulfuron (Glean ®) application in both field and glasshouse experiments.

Cropping pattern also has a considerable impact on this disease. Rovira (1986) reported consistently larger areas of affected crop when wheat followed a mixed annual pasture of grasses and *Medicago* spp. *R. solani* isolated from rotted wheat roots could produce severe cortical rot and brown-tipped truncated roots on wheat, barley, peas, *Medicago* spp., annual rye (*Lolium rigidum),* barley grass (*Hordeum glaucum*), as well as lentil. Smith & Wehner (1989) found in a three year field study of fallowing in crater diseased soil, or cropping with sunflower, maize, grain sorghum, soybean or cotton, that the fungus could neither be isolated from, nor it could infect, any crops other than wheat. Root exudates from wheat and grain sorghum stimulated hyphal growth of *R. solani* to a significantly greater extent than root exudates from the other crops.

Calcareous sandy loam is the soil type most frequently reported to be favourable for *Rhizoctonia* bare patch (Samuel & Garrett, 1932). The disease is also found in white neutral-acid siliceous sands, water repellent siliceous sand, red brown earths and other soils with poor fertility (Dubé *et al.*, 1987). There have been some reports of disease reduction when nitrate fertilizer was applied (Chambers, 1966) but MacNish (1988) found that nitrogen applications had no effect on the incidence of *Rhizoctonia* root rot.

As *R. solani* can infect most plant species, this prevents the use of crop rotation to control the disease. In the field, Rovira (1986) reported infection in wheat, barley, peas, *Medicago* spp., annual rye grass and barley grass as well as lentil. In controlled-environment experiments comparing different cereal species and cultivars, Neate (1989) found that wheat had the highest disease rating, followed by oats, barley, triticale and rye, but in the field barley had the highest rating followed by wheat and then oats. The

overall differences between the cereals were small.

The effect of seasonal variation on disease severity has not been closely studied. However, it was observed that a dry and cold period after autumn rainfall seemed to accelerate disease severity (Anon., 1987).

Baker & Martinson (1970) suggested two possible conditions which might increase the disease potential. Firstly, the immobilization of essential plant nutrients by the fungus may result in mineral deficiencies in the host plant (Alexander, 1961). Secondly, the formation of phytotoxic compounds during decomposition of the organic residues (Cochrane, 1948; Patrick & Koch, 1958) may affect plant metabolism directly or increase the exudation of a substance stimulating the pathogen (Toussoun & Patrick, 1963).

II.8. CURRENT CONTROL MEASURES

At present, the only effective way to control this disease is by two or more tillage operations before sowing (Neate, 1985; Pumphrey *et al.*, 1987; MacNish & Fang, 1987; Smith & Wehner, 1988). Roget *et al.* (1987) suggested short 'chemical fallow', or spraying herbicide some weeks before sowing to kill the grass pasture before direct-drilling might reduce the *Rhizoctonia* inoculum, but Rovira & McDonald (1986) found that herbicides such as chlorsulfuron increased the damage caused by this disease and decreased wheat yield. Other control strategies such as fungicides (Cotterill *et al.*, 1989; Cotterill & Ballinger, 1989, Smiley *et al.*, 1990), breeding for resistance (Purss and Dubé, 1984) and alternative cropping systems (Rovira, 1986) have proven to be unsuccessful. Multiple cultivations using wide tynes cause significant soil disturbance, probably disrupting the network of fungal hyphae in the surface soil (Neate, unpublished data). Some wheat growing areas in South Australia and Victoria have sandy soils, which are easily eroded under frequent 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* bare patch (Samual & Garrett, 1932) and

minimum tillage has been associated with this increase in disease severity (Rovira, 1986).

The results mentioned above suggest that only partial control is achieved currently, and an integrated management approach involving tillage, chemical fallow and nutrition might be required.

PART III : ZN AND DISEASE INTERACTIONS

The literature on the effects of the Zn nutritional status of the host plant on resistance to diseases is still perplexing. According to reviews on this subject by Graham (1983), Huber (1989) and Graham & Webb (1991), Zn affects numerous diseases in both promotive and inhibitory manners, as shown in Table I.1. However, effects of Zn on *Rhizoctonia* root rot have not been reported so far.

Zn has been reported to stimulate germination of fungal spores but whether this effect of Zn is significant in pathogenesis generally is not known (Graham, 1983). On the other hand, numerous studies have also reported that the suppressive effect of zinc on disease is due to the direct toxicity of high zinc on the pathogen, for example, Wilkinson & Millar (1981), Somashekar *et al.* (1983), Cripps *et al.*(1983), Hooley & Shaw (1985). Most of these experiments were carried out in agar media, the rates of Zn used ranging from 1 to 50 mg Zn/L, indicating that Zn had toxic effects directly on the pathogens. The effects of these levels of Zn on plant growth, which are likely to be too high for plant growth, were certainly not considered. Prasad (1979) reported that Zn at 3 mg/L in culture solution checked the symptoms of *Fusarium oxysporium* var. *lini* on flax but did not stop the spread of the fungus through the production of the toxin, fusaric acid. In soil-plant systems, the use of Zn in high concentration as a fungicide to control diseases by adding soil soaked with Zn (Haque & Mukhopadhyaya, 1983), spraying (Savor, 1986) or dipping the seeds in a Zn solution before planting (Somani, 1986), was reported. No effects and reports on the nutritional status of Zn in the host plant were considered.

Table I.1 List of plant diseases influenced by Zn (Huber, 1989).

Disease	Pathogen	Host Plant	Effect of Zinc
Bacterial Disease			
Bacterial Blight	*Xanthomonas campestris pv oryzae*	Rice	Increase
Fungus Diseases			
Leaf spot	*Helminthosporium* spp.	Barley	Decrease
Root rot	*Fusarium culmorum*	Cereals	Decrease
Root rot	*Helminthosporium sativum*	Cereals	Decrease
Wilt	*Verticillium dahliae*	Cotton	Decrease
Leaf spot	*Drechslera siccans*	Lolium	Increase
Fruit rot	*Penicillium italicum*	Mandarin	Increase
Root rot	*Phytophthora nicotanae var parasitica*	Mandarin	Decrease
White rot	*Sclerotium cepivorum*	Onion	Decrease
Root rot	*Aphanomyces cochlioides*	Pea	Decrease
Wilt	*Fusarium udum*	Pigeon Pea	Decrease
Late blight	*Phytophthora infestans*	Potato	Decrease
Storage rot	*various*	Potato	Decrease
Sheath rot	*Sarocladium oryzae*	Rice	Decrease
	Oidium	Rubber	Decrease
Covered smut	*Sphacelotheca sorghi*	Sorghum	Decrease
Leaf spot	*Cercospora* spp.	Sugar beets	Increase
Rust	*Puccinia melanocephala*	Sugar cane	
Leaf mould	*Corynespora* spp.	Tomato	Decrease
Late blight	*Phytophthora infestans*	Tomato	Decrease
Leaf mould	*Pseudocercospora fuligena*	Tomato	Decrease
CLS virus	*Spongospora subterranea* f. sp. *nasturtii*	Watercress	Decrease
Stem rust	*Puccinia graminis*	Wheat	None
Leaf rust	*Puccinia recondita*	Wheat	Decrease
Leaf rust	*Puccinia recondita*	Wheat	Increase
Rust	*Puccinia* spp.	Wheat	Decrease
Rust	*Puccinia* spp.	Wheat	Increase
Stripe rust	*Puccinia striiformis*	Wheat	None
Loose smut	*Ustilago tritici*	Wheat	Increase
Virus Diseases			
Mosaic	*Tobacco mosaic virus*	Bean	Increase
Mosaic	*Tobacco mosaic virus*	Nicotiana glutinosa	Decrease
Mosaic	*Tobacco mosaic virus*	Tobacco	Decrease
Mosaic	*Tobacco mosaic virus*	Tobacco	Increase
Virus	*Chlorotic Leaf spot*	Watercress	Decrease

There has not been much work involving levels of Zn near deficiency. The report of Millikan (1938) seems to be a pioneer, in which he observed that more severe root-rot diseases occurred when wheat was grown in the Zn-responsive soil without Zn applied, compared with wheat supplied with 35 kg/ha of $ZnSO_4$. $7H_2O$. However, the concentration of Zn in those plants were not reported. Bolle-Jones & Hilton (1956) showed more *Oidium heveae* infection in Zn-deficient rubber leaves, and Reis *et al.* (1982) showed that take-all infection of wheat plants was suppressed by adding Zn in culture solution, but there has so far been no explanation for the mechanism involved. The only researcher who has approached an explanation for the Zn suppression mechanism since that time was Tomlinson (1958) who showed that supra-optimal Zn supply, at the rate of 0.5 mg/kg, could suppress crook root in watercress by inhibiting the growth of the pathogen, *Spongospora subterranea.* Later, Tomlinson & Hunt (1987) found that the suppression effect of Zn on *Spongospora subterranea* , the vector of watercress chlorotic leaf spot virus (WCLV), inhibited the symptoms of chlorotic leaf spot, and so explained the decrease of this disease in watercress. Sparrow & Graham (1988) demonstrated that Zn application of 0.06 mg/kg soil could reduce crown rot disease in cereals by slowing the upward progression of the pathogen, *Fusarium graminearum* through the stele of the wheat plant.

Evidence from all those studies in the deficient range of Zn suggests that effects of Zn on disease suppression may be through physiological processes that predispose the plant to disease infection. The explanation of how Zn could suppress certain diseases is yet to be clarified. The hypothesis of Zn-deficiency-induced membrane leakage increasing promotive root exudates to stimulate the pathogen's invasion was suggested by Sparrow & Graham (1988) from their studies mentioned above.

Studies on the effect of mineral nutrients on diseases might comprise (i) observation of an association between mineral status and disease incidence or severity, both in the field and pots, by means of correlation, (ii) observation of an effect of increasing rate of inoculum and, in particular, the effects of nutrient levels ranging from

sub-optimal or deficiency to supra-optimal, on disease severity, and (iii) a separate study on the effects of nutrients on pathogens and host plants to identify mechanisms of the cause-and-effect relationship between the nutrient and disease. Points to be considered in this type of study have been suggested by Colhoun (1973), Graham (1983) and Huber (1989).

CHAPTER 2

PRELIMINARY FIELD OBSERVATIONS ON THE ZN-DISEASE INTERACTION

2.1. INTRODUCTION

To justify a detailed study of the interaction between disease and an environmental factor it is desirable to establish evidence of a relationship between the particular disease and factor under field conditions (Colhoun, 1973). Except for the report of Millikan (1938), which was only a simple observation on the yield response to applied Zn of cereals infected by a group of root rot pathogens, of which *R. solani* was one, no direct evidence of the association between *Rhizoctonia* root rot disease and Zn nutritional status of cereals was found in any literature at the beginning of this project. Thus, further evidence of this relationship needed to be investigated.

In order to achieve this objective, two field studies were conducted in cereal growing areas where *Rhizoctonia* root rot and Zn deficiency were known to occur. The first experiment was a paddock survey in 1989; the other is part of the long-term Zn experiment of Dr R J Hannam, South Australia Department of Agriculture, in which our measurements were made in 1989 and 1990.

2.2. EXPERIMENT 2.1: PADDOCK SURVEY TO STUDY THE CORRELATION OF ZN CONCENTRATION IN CEREAL TOPS AND SEVERITY OF DISEASE INFESTATION, 1989

2.2.1. MATERIALS & METHODS

2.2.1.1 *Site description.* A farmer's field at Lameroo, 200 kms southeast of Adelaide, South Australia, was used for this study. The field, with siliceous sandy soil, was reported to have poor Zn nutritional status. A nutrition survey in 1986 reported that

the Zn concentration in the youngest fully-emerged leaf blades (YEB) of wheat in this field was 10 mg/kg (Hannam, 1991), much below the desirable minimum of 20 mg/kg. The average annual rainfall is 410 mm, and the total rainfall in 1989 was 338 mm. The field was in the wheat phase of a grassy pasture/wheat rotation (for the last 15 years) and *Rhizoctonia* root rot was reported to be widespread.

2.2.1.2 *Field preparation.* An area of 42 ha was sown to wheat (*Triticum aestivum* cv Spear) by direct-drill, with an application of 55 kg/ha of diammonium phosphate. All the preparations followed the farmer's common practice and were carried out by the farmer.

2.2.1.3 *Plant Sampling and Processing.* By 4 weeks after sowing, when plants were at the 3-leaf to tillering stage, areas of bare patch became obvious and plants were randomly sampled from 60 points all over the field, as mapped in Figure 2.1. Each spot was categorized as either inside or outside a bare-patch area before approximately 10 plants, with roots and shoots, were collected in one spade-full. The roots of each individual plant were separated from the shoots, put in a plastic bag and, after cleaning, were assessed for disease severity, expressed as a percentage of seminal roots infected (%SRI) as described by McDonald and Rovira (1985). Shoots were put in a separate bag, dried in the hot air oven at 65°C for at least 48 h before being measured for dry weight, digested according to Zarcinas (1984) and analysed for Zn concentration by Inductively Coupled Plasma Optical-Emission Spectrometer (ICP). All statistical analyses used the GENSTAT 5 program (GENSTAT 5 Committee, 1989).

2.2.2. RESULTS

Of the total of 60 recorded samples, 40 fell inside bare patches and 20 outside. Shoots of plant samples collected from inside and outside patches are shown in Plate 4. The distribution of total samples inside and outside bare patches was found to conform to a binomial distribution (Zar, 1984), which confirmed that the number of samples was appropriate to this study (Appendix 1). Of the 40 samples inside patches, 16 were

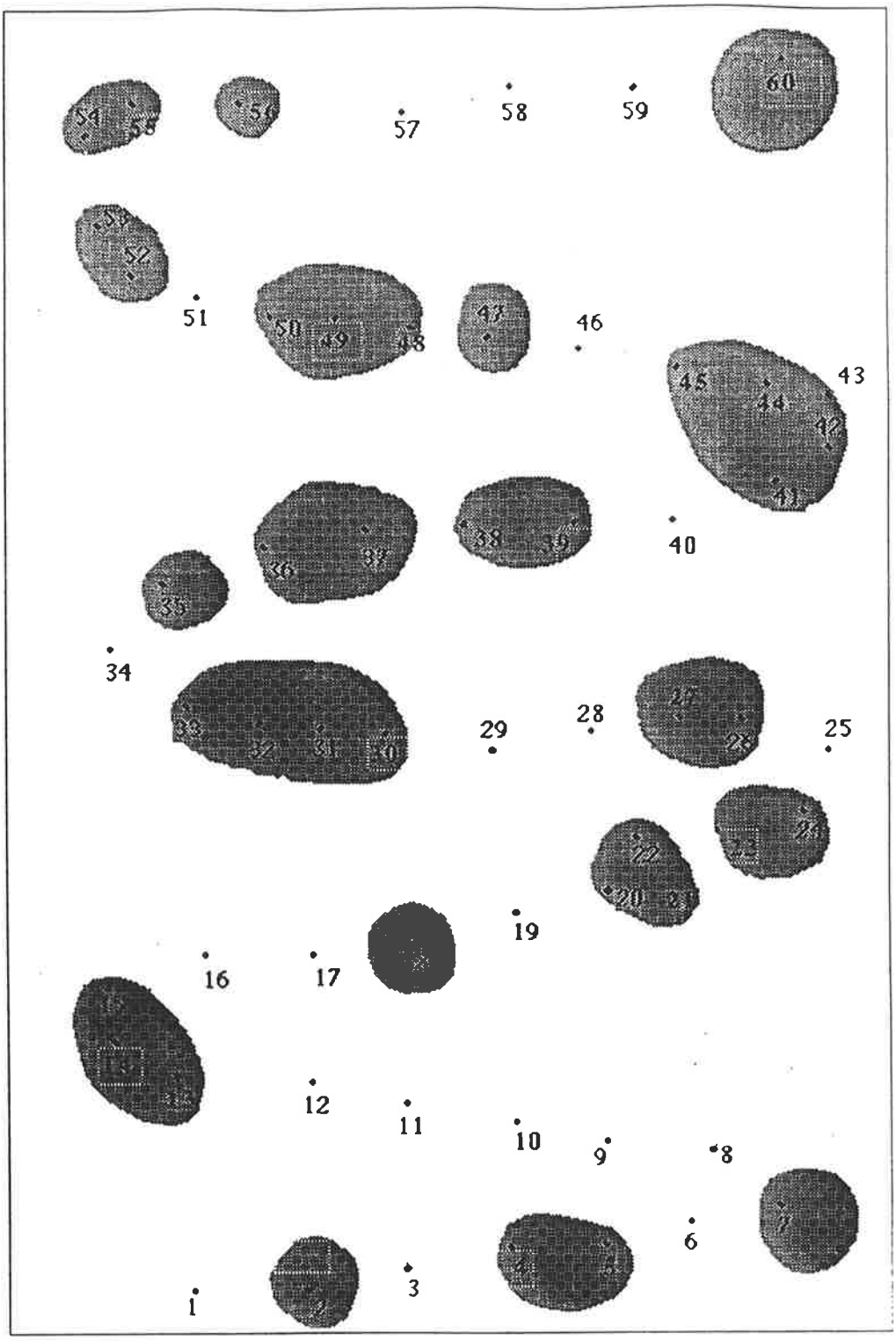


Figure 2.1 Diagram showing sampling points in the farmer's field at Lameroo, Experiment 2.1, 1989. The total area of 42 ha was sown to Spear wheat. The shaded areas represent bare patches.

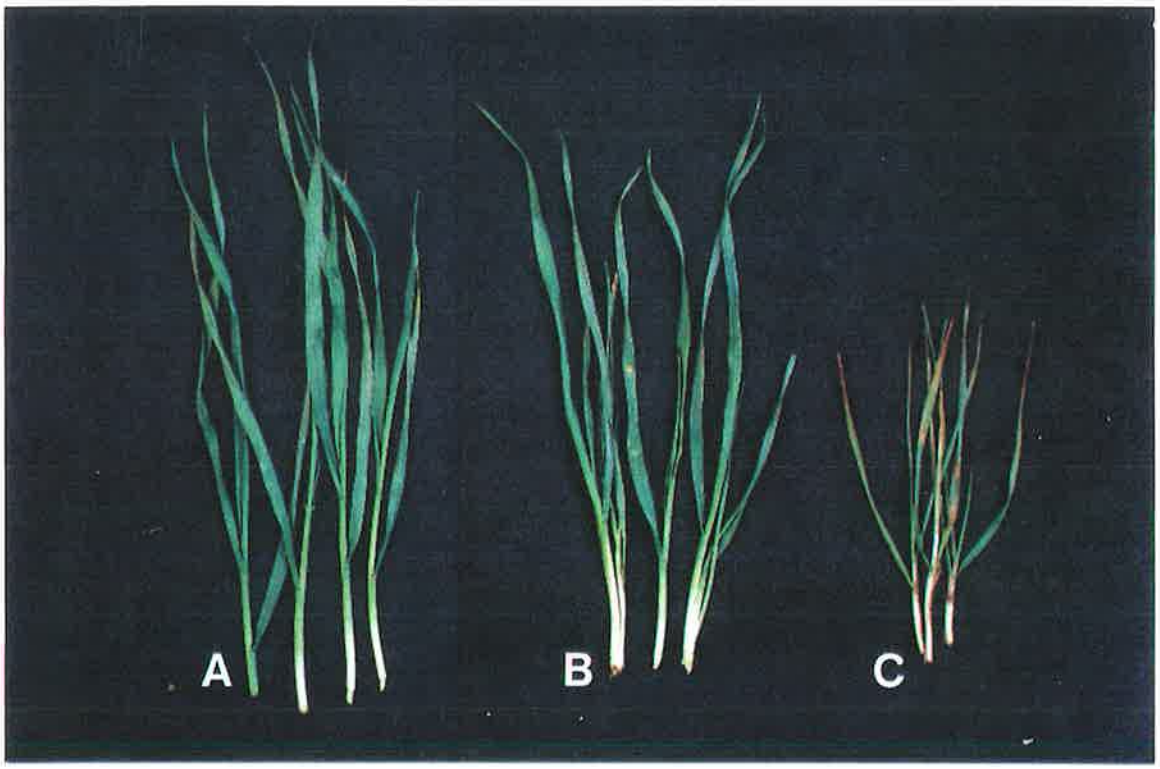


Plate 4 Shoots of Spear wheat plants collected from Experiment 2.1 at Lameroo, conducted in a farmer's field, 1989.

A. Healthy plants from outside a bare patch.

B. Fair plants from outside a bare patch but affected by Zn deficiency.

C. Stunted plant from inside a bare patch.

severely infected by cereal cyst nematode (CCN) as well as *Rhizoctonia* root rot and these were excluded from the correlation.

Multiple correlation showed that none of the nutrient elements in the plant tops interacted with each other in their association with disease severity (Appendix 2), so simple linear correlations were calculated for the relationships between %SRI and the concentration of each individual element. The correlation matrix in Table 2.1 shows that Zn had the highest and the only significant association with disease severity measured in terms of %SRI (r=-0.38*).

A curvilinear correlation of disease severity with Zn concentration in shoots was calculated in order to further characterise the relationship (Figure 2.2). Disease severity, expressed as %SRI, was significantly correlated to the reciprocal of Zn concentration in plant tops (R^2=0.52**). Zn concentration ranged from as low as 4.5 up to 20 mg/kg, which is still considered to be in the marginal deficiency range. %SRI ranged from as high as 80% to around 20%, with a sharp drop as Zn concentration in the plant top increased from 5 to 10 mg/kg.

The distribution of dry weight of plant tops at each sampling point according to its Zn concentration and %SRI is shown in Figure 2.3. Top dry weight of plants tended to decline with both the increase of %SRI and the decrease of Zn concentration. Dry weight ranged from 0.03 g/plant at 90% infection to 0.24 g/plant for low infection. The low dry weights within the range of 20% infection may be explained either by Zn deficiency without infection, or possibly by CCN infection or some other independent limiting factor.

Table 2.1 Correlation coefficients between the concentration of nutrient elements in 60 individual wheat shoots and disease severity on the roots expressed as percent seminal root infected (%SRI). Experiment 2.1, Lameroo, 1989. The value with * is significant at $p<0.05$.

Nutrient	Correlation coefficient (r)
Fe	-0.007 ns
Mn	-0.230 ns
B	-0.265 ns
Cu	-0.134 ns
Mo	-0.113 ns
Co	-0.115 ns
Ni	-0.035 ns
Zn	-0.380 *
Ca	0.047 ns
Mg	-0.002 ns
Na	-0.201 ns

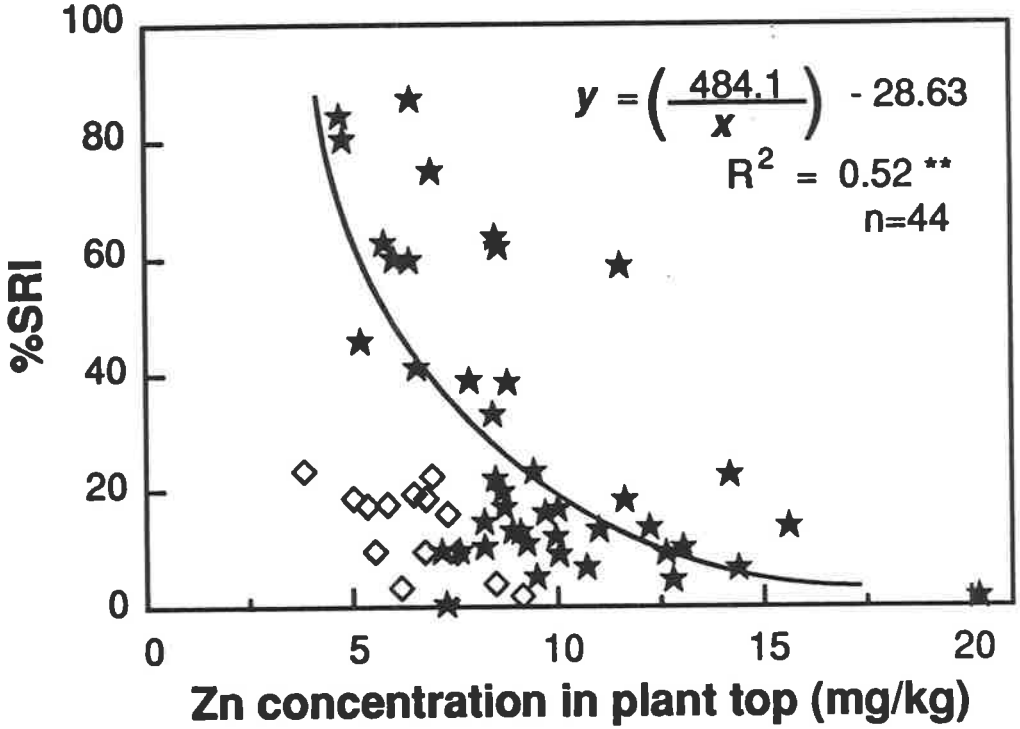


Figure 2.2. Curvilinear correlation between Zn concentration in plant tops and disease severity, in terms of % seminal roots infected (%SRI), Experiment 2.1, 1989. ★represents 44 points used in the calculation and ◇ represents 16 sampling points severely infected by CCN and excluded from the correlation.

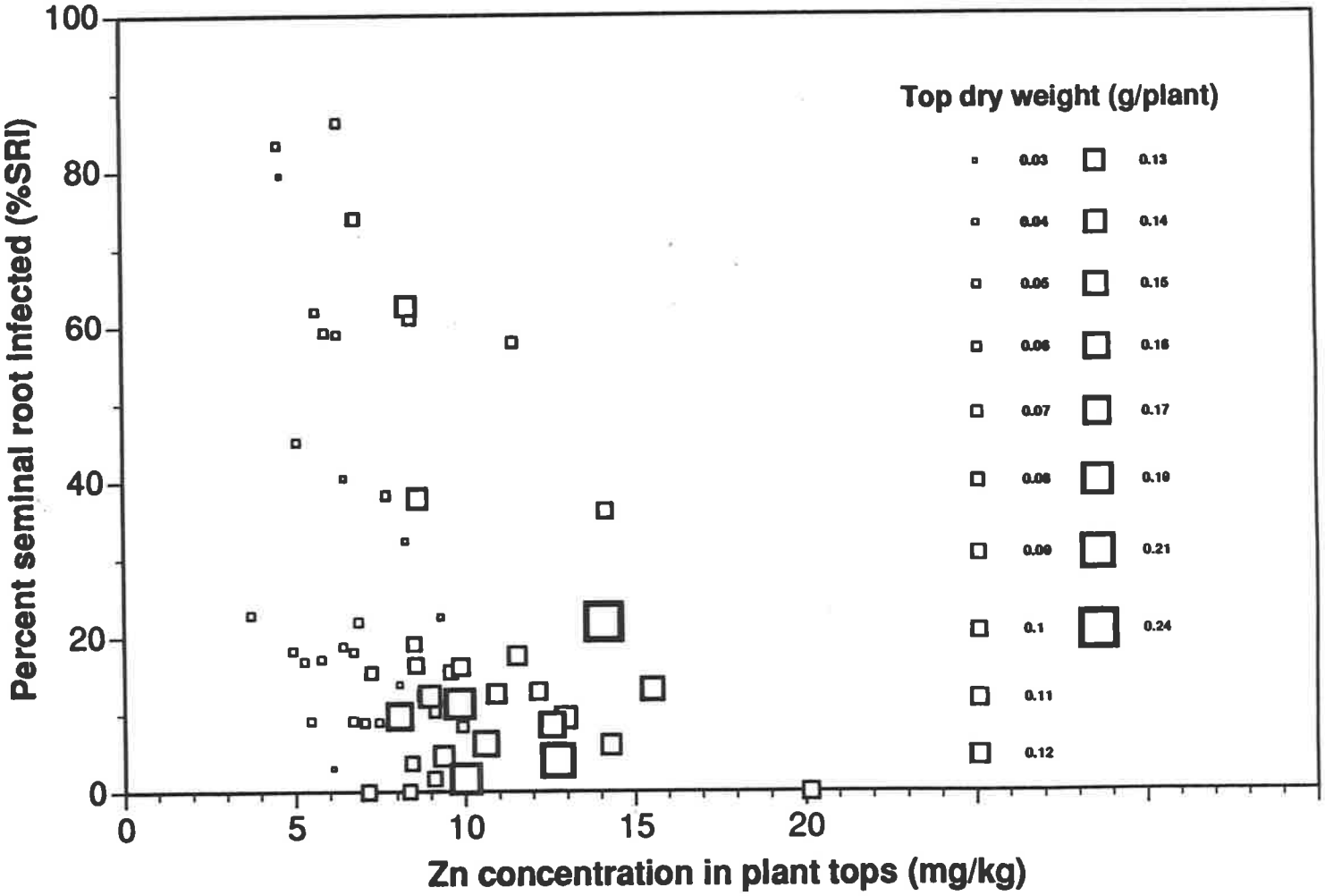


Figure 2.3 Plant top dry weight at each sampling point corresponding to its Zn concentration and % seminal roots infected.

2.3. EXPERIMENT 2.2 : EFFECT OF ZN APPLICATION TO SOIL ON SEVERITY OF *RHIZOCTONIA* ROOT ROT IN CEREALS GROWN IN THE FIELD, 1989 & 1990.

This long-term experiment has been conducted since 1987 as part of the Waite Institute-South Australia Department of Agriculture (SADA) collaborative programme surveying the nutrition of cereal crops in the Murray Mallee. Results of that survey indicated how widespread severe Zn deficiency was in crops of this region during the period of 1986-1988 (Graham *et al.*, 1990). The experiment was the responsibility of Dr. R J Hannam of SADA, and aimed to diagnose criteria for determining the Zn status of some field and pasture crops, to examine the efficiency of methods of applying Zn fertilizer to soil, and also to examine the residual effect of Zn fertilizer applied in those soils. However, it appeared that *Rhizoctonia* bare patch started to become widespread in the experimental plot area in 1988 and even more severe in 1989, so this experiment was suitable for our purpose to study the effects of Zn applied to soil on severity of *Rhizoctonia* root rot. All of the experimental plans and field preparations were done by Dr R J Hannam and staff of the SADA.

2.3.1. MATERIALS & METHODS

2.3.1.1 *Site description.* Another farmer's field at Lameroo, 5 kms from the field of Experiment 2.1, has been used for a long term Zn trial since 1987. In 1986, Zn concentrations of 9 mg/kg in YEB were measured in wheat on this site (Graham *et al.*, 1990). The site was confirmed to be free of cereal cyst nematode as no eggs were found in the soil before planting each year.

2.3.1.2 *Experimental design.* In 1987, a long-term experiment was established in a randomized complete block design with 4 replicates. Each replicate contained eleven plots, 20 m long and 2 m wide, with Zn applied at rates of 0, 0.1, 0.25, 0.5, 1, 2.5, 5, 7.5, 10, 15 and 20 kg Zn/ha. In 1990, another five previously untreated

plots, reserved in each replicate for this purpose, were supplied with Zn at rates of 0.5, 1, 2.5, 5 and 10 kg Zn/ha. All plots were otherwise managed similarly.

2.3.1.3 *Zn application.* Zinc solutions were prepared by dissolving $ZnSO_4.7H_2O$ in double deionized water to the desired concentration. The solution was applied to the soil surface on the appropriate plot using a boom spray attached to a cart fitted with a plastic curtain to prevent Zn contamination of the adjacent plots. The Zn was incorporated to a depth of 10 cm by two passes of a rotary hoe, starting from the nil Zn plots and proceeding progressively to the highest rate. The rotors of the rotary hoe were cleaned between treatments by rotary hoeing unfertilized soil outside the experimental area. All plots were rotary-hoed to ensure a common tillage treatment.

In 1988, the field was sown to Paraggio medic (*Medicago truncatula* cv Paraggio) after one working up of each plot with a tyned tillage implement and application of triple super phosphate at the rate of 30 kg P/ha. Significant, severe *Rhizoctonia* bare patch was observed in the medic crop.

2.3.1.4 *Field preparation.* In 1989, the site was prepared by two workings with a tyned tillage implement and sprayed with glyphosate at 1.7 litres/ha to ensure a clean seed-bed. Two weeks after preparation, barley *(Hordeum vulgare L.* cv Galleon) was sown at 55 kg/ha with applications of diammonium phosphate, NH_4NO_3 and Mn as $MnSO_4$ to result in rates of 90 kg N/ha (split application), 20 kg P/ha and 17 kg Mn/ha.

In 1990, all practices were similar to the previous year except that fresh Zn was applied to untreated plots as previously described, and the field was sown to wheat (*Triticum aestivum* L. cv Spear).

2.3.1.5 *Bare patch assessments.* In 1989, when barley plants were at the heading stage, the area of bare patches was mapped in all replications of each treatment. A patch was identified as an area which had smaller plants with fewer tillers than surrounding areas. The areas of bare patches were drawn on a map of the experiment. Areas of the patches were cut from the map and the remaining part of that map or

uninfected area of the plot was passed through a leaf area planimeter and compared with the complete plot. The percentage of the area affected by bare patches (%BPA) was then estimated.

In 1990 greater accuracy was sought by superimposing a string grid of 1 m^2 on each 20 x 2 m plot of wheat at early tillering in order to draw the map of bare patch areas on graph paper; the %BPA was assessed as before. Each 1 m^2 grid area was also observed for the presence of bare patch, and the number of the grids with bare patch in each plot was used to calculate the percent of patch appearance (%PA) for each plot. This method is modified from the patch scoring method (MacNish & Lewis,1985) in which the patches were scored every 1m for each row of plant.

2.3.1.6 *Assessment of root infection* In 1989, severity of disease on plant roots was not assessed as %SRI because it was difficult to get the complete root system at the observed growth stage owing to the very dry soil.

In 1990, one plant was collected from the centre of every second grid in each treatment, giving a total of 20 plants per plot. The shoot of each plant was separated from the root, folded into a small bundle using waxed weighing paper, put in a separate bag and dried in the oven for dry weight and elemental analysis as described in the previous experiment. The root was washed and classified as *Rhizoctonia* infected or uninfected according to the visual symptom of spear tips and/or cortical truncations in the root system. Percent of disease incidence (%DI) for each plot was then calculated from the number of infected plants per plot. The percentage of seminal roots infected, or %SRI, was also assessed as previously described. The four methods of disease severity assessment used in 1990, as summarized in Table 2.2a, were then correlated as shown in Table 2.2b. The methods of assessing disease severity in the field compared percent bare patch area (%BPA), percent patch appearance (%PA), percent disease incidence (%DI) and percent seminal roots infected (%SRI) were not significantly correlated to each other. Percent bare patch area (%BPA) was then used as in 1989.

The causal fungus was isolated from some root samples in each patch by the modified method explained by Neate (1984). Parts of the roots with the symptoms of either spear tip or cortical truncations were cut approximately 0.5 cm above the symptom, washed twice in sterile Milli-Q® water, dipped in 70% ethanol for 30 sec. then again washed twice in sterile water. After blotting dry with the sterile filter paper, the pieces of root were placed on weak Czapek Dox plus yeast extract agar medium (1/6NDY: Appendix 3) containing 100 mg/l streptomycin sulphate and 50 mg/l tetracycline hydrochloride. Plates were incubated at 15°C and inspected for emerging hyphae using a binocular microscope several times between 18 and 48 h after plating. This confirmed the presence of *R. solani* (Plates 5 & 6).

2.3.2. RESULTS

In 1989, there was a sharp decline in %BPA from 42% in the nil Zn treatment to 21% when 2.5 kg Zn/ha was applied, and then a further gradual decline to 13% at 20 kg Zn/ha (Figure 2.4). The correlation between the reciprocal of Zn applied and %BPA was highly significant (R^2=0.90**). Zn concentration in YEB was also reciprocally correlated to disease severity in terms of %BPA (Figure 2.5), in the same manner as Zn concentrations correlated with %SRI in Experiment 2.1.

Results presented in Table 2.3, show that applying Zn significantly increased grain yield from 1.2 t/ha in the plot without Zn to 1.59 t/ha in the plot with 0.1 kg Zn/ha. It was at the rate of 2.5 kg Zn/ha that yield was increased up to 2.54 t/ha, a figure which was not significantly different from that of the other five higher rates of Zn: 5, 7.5, 10, 15 and 20 kg/ha, where a highest yield figure of 2.8 t/ha was achieved. This response underlies a negative correlation between grain yield and %BPA (Figure 2.6) which is also highly significant (R^2=0.80**).

In 1990 there was a long dry period at the beginning of the season and sowing was delayed well past the optimum date. Nevertheless, in this season, high rates of Zn appeared to decrease %BPA from 40% in the nil and low Zn treatments, to around 20%

Table 2.2a Summary of indices for severity of *Rhizoctonia* root rot disease used in the long-term Zn experiment at Lameroo. Experiment 2.2, 1990.

Disease severity index	Abbreviation	Method
Percent bare patch area	%BPA	The hand-drawn maps of the bare patch area.
Percent patch appearance	%PA	Percentage of grids (each of 1 m^2 area) with bare patches present, based on a total of 20 grids per plot
Percent disease incidence	%DI	Percentage of plants with root infection based on a total sampling of 20 plants per plot
Percent seminal roots infected	%SRI	Percentage of seminal roots with spear tips or cortical truncations based on total seminal roots of each plant, averaged over a sampling of 20 plants per plot

Table 2.2b Matrix of correlation coefficients (r) among all disease severity indices as described in Table 2.2a, Experiment 2.2, Lameroo, 1990. None was significant so %BPA was used to be consistent with what was used in the same experiment in 1989.

	% DI	% PA	% BPA	% SRI
% DI	1			
% PA	0.07	1		
% BPA	-0.25	0.24	1	
% SRI	-0.15	-0.19	-0.01	1

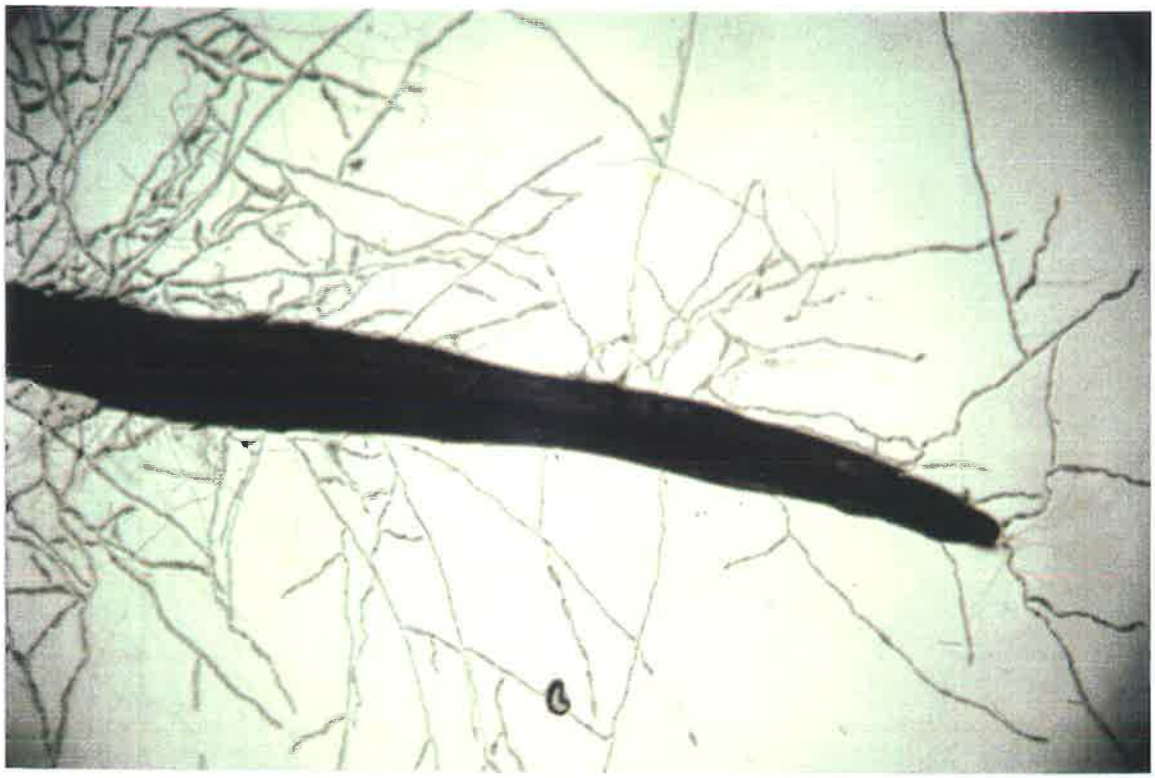


Plate 5 Mycelium of *Rhizoctonia solani* growing from a root of an infected barley plant from Experiment 2.2, Lameroo (x10).

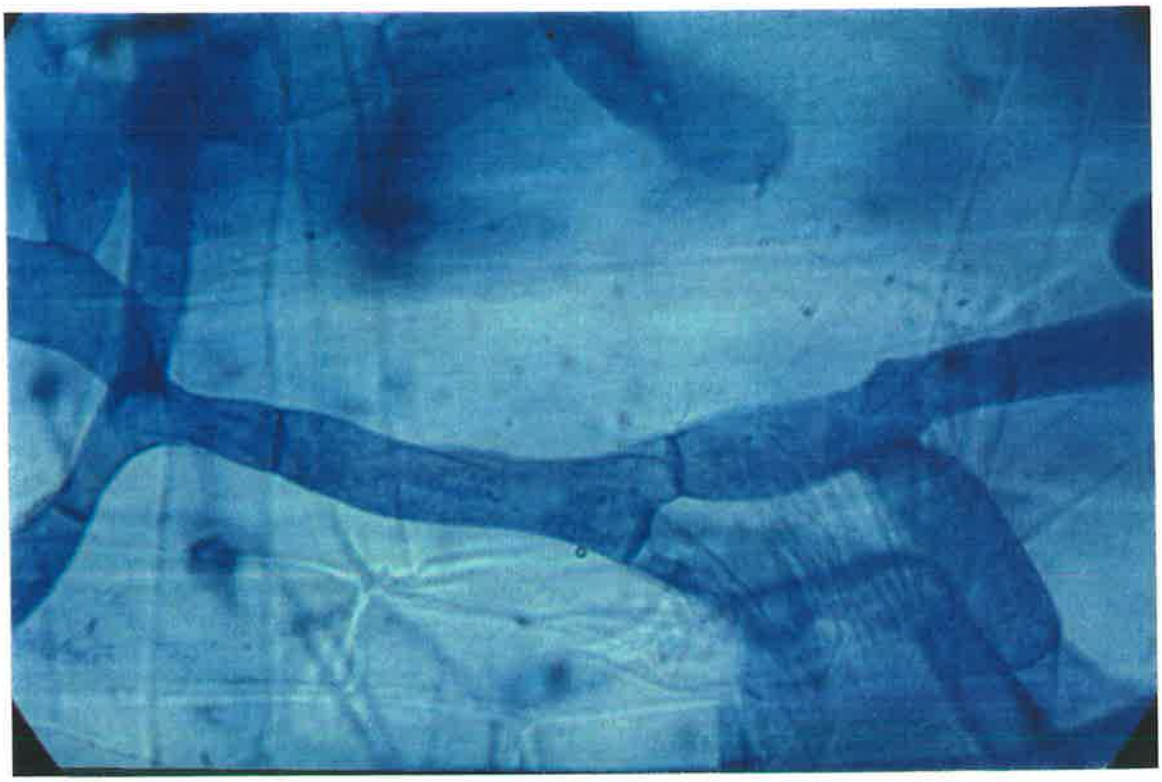


Plate 6 Mycelium stained from Plate 5, stained with Trypan Blue and confirmed to be *Rhizoctonia solani* (x100).

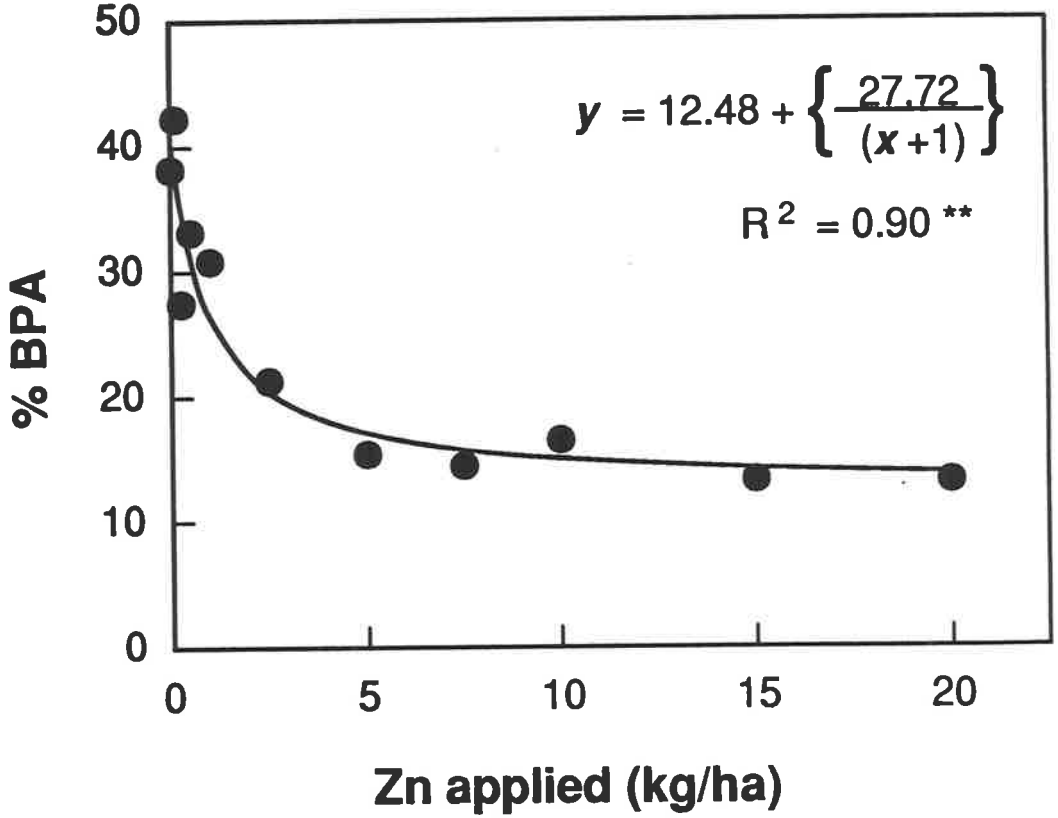


Figure 2.4 Curvilinear correlation between Zn applied (kg/ha) and mean percent bare patch area (%BPA) of each Zn treatment, Experiment 2.2, 1989.

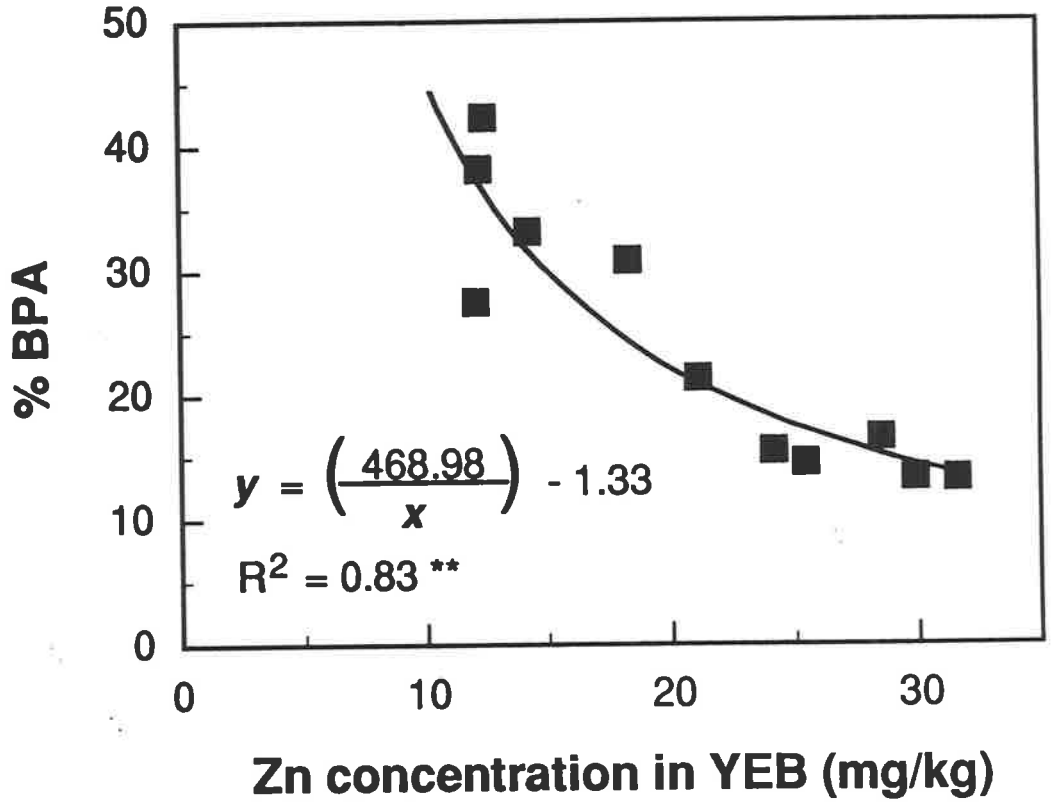


Figure 2.5 Correlation between Zn concentration in the youngest fully emerged leaf blade (YEB) and percent bare patch area (%BPA), Experiment 2.2, 1989.

Table 2.3 Grain yield (t/ha) in 1989 and 1990 from the field experiment 2. For 1989, yields followed by the common letter are not different at p>0.05 compared by Duncan's Multiple Range Test. For 1990, yields are not significantly different. Unpublished data of Dr R J Hannam.

Zn applied (kg/ha)	**Grain yield in 1989** (t/ha) Zn applied in 1986	**Grain yield in 1990** (t/ha) Zn applied 1986	Zn applied 1990
0	1.12 d	1.30	1.30
0.10	1.59 cd	1.31	-
0.25	1.72 bc	1.41	-
0.50	1.77 bc	1.22	1.31
1.00	2.23 ab	1.35	1.37
2.50	2.54 a	1.42	1.31
5.00	2.55 a	1.40	1.30
7.50	2.80 a	1.43	-
10.00	2.75 a	1.23	1.40
15.00	2.61 a	1.33	-
20.00	2.47 a	1.23	-
		ns	

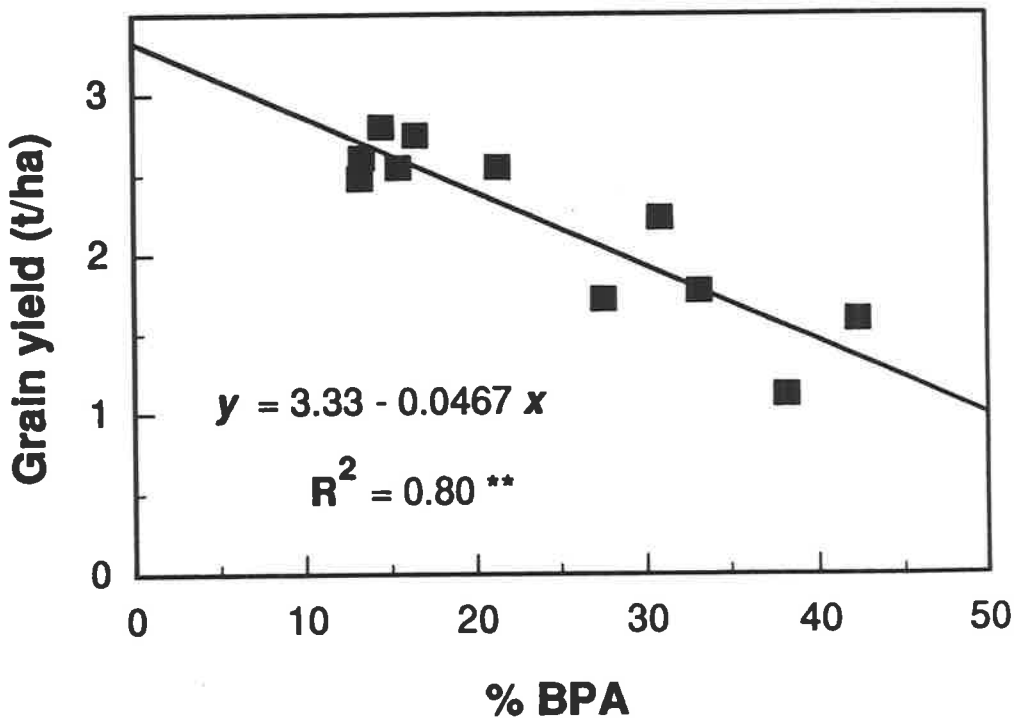


Figure 2.6. Correlation between grain yield of Galleon barley (t/ha) and %BPA, Experiment 2.2, 1989.

at the highest Zn rate, although the relation was not statistically significant (Figure 2.7). There is a trend of more severe disease in treatments where Zn was applied in 1986 than in corresponding treatments where Zn was applied in 1990; %BPA of the former was approximately 5-10% higher than the latter, however, this difference is not significant. There was also no significant response of grain yield to Zn treatments according to the analysis of variance (Table 2.3).

2.4 DISCUSSION

2.4.1. CORRELATION BETWEEN DISEASE SEVERITY AND ZN STATUS OF THE PLANTS.

This is the first report of a relationship between Zn nutritional status and susceptibility of cereals to *Rhizoctonia* root rot. The specificity of the Zn effect is shown by the much higher correlation coefficient between Zn and disease severity than was the case for any other element analysed in the first study. Results from the following experiment, where disease severity was decreased according to the increase in Zn application, supported the result from the survey experiment. The pattern of inverse correlations between disease severity and Zn status, which was confirmed in both field experiments, indicated that disease severity decreased proportionally to the increase of Zn concentration in the plant tops, up to the critical concentration of Zn.

No definitive evidence like this has been reported before. Millikan (1938) reported a yield response in cereals to Zn application and observed that there appeared to be less infection in the Zn-treated plots, but it was not only *R. solani* that might have been responsible as a causal pathogen and neither the Zn status of the plants nor its effect on disease severity was measured. Somani (1986) reported an effect of Zn on decreasing potato black-scurf caused by *R. solani* of a different anastomosis group, but used high concentrations of Zn as a fungicide to treat tubers before planting. There are some reports on the effects of Ca, N, P and K in decreasing root rot disease caused by *R. solani* when nutrition was varied over the range from deficiency to adequacy (Huber,

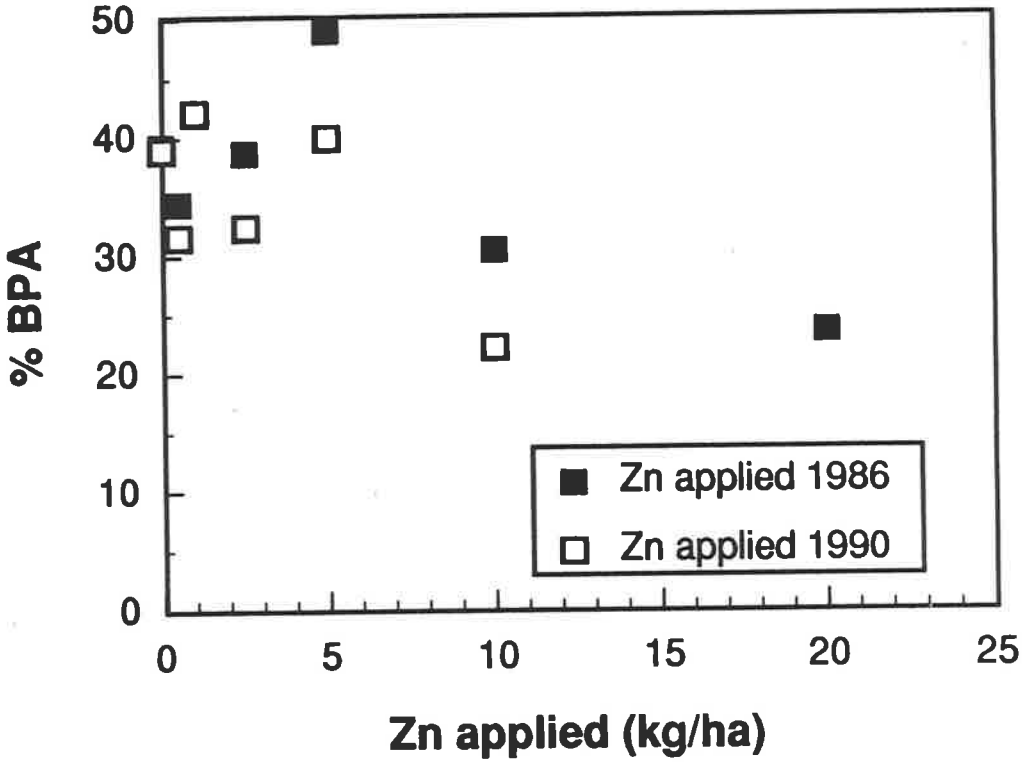


Figure 2.7. The nonsignificant correlation between Zn applied (kg/ha) and percent bare patch area (%BPA) in Spear wheat, Experiment 2.2, 1990. ■ represents %BPA from the plots supplied with Zn in 1986 and □ represents %BPA from plots supplied Zn in 1990.

1991; Srihuttagum & Sivasithamparam, 1991). However, in our experiments these nutrients were kept at adequate levels, thus eliminating confounding effects on severity of the disease.

2.4.2. IMPLICATIONS OF THE ZN-DISEASE INTERACTION FOR CROP HUSBANDRY.

The sharp change in slope of the curve of %BPA vs Zn applied at the level of 2.5 kg Zn/ha in 1989 is useful information, since this is the recommended rate of Zn application in the Mallee and Eyre Peninsula areas of South Australia where not only *Rhizoctonia* root rot, but also Zn deficiency, are serious problems (Hannam, 1990). This point will be discussed in more detail in the general discussion section.

2.4.3. SEASONAL VARIATION.

Marked seasonal variation in the intensity of Zn deficiency is characteristic of the South Australian environment (Graham, 1991). It is not uncommon that a field trial will show strong grain yield responses one year, and on the same site no response the next. This happened in the pair of years 1989 and 1990. Responses to Zn in growth and grain yield were large and highly significant in 1989 but non-significant in 1990. The diminished effects of Zn in 1990 may be associated with a long dry period at the beginning of the season which delayed sowing; the late sowing or associated effects were unfavourable to the development of Zn deficiency and its consequences. The magnitude of the Zn effect on disease severity was greater in 1989 than in 1990. The lack of significance in the correlation between %BPA and Zn concentration in plant tops, between %BPA and grain yield, or between grain yield and rate of Zn applied (Table 2.4) may have been because of the unfavourable start to the season.

These results, on the other hand, highlight the fact that the effects of Zn in decreasing disease severity must have been through physiological processes affecting

Table 2.4. Matrix of correlation coefficients (r) between Zn applied (kg/ha), Zn concentration in YEB (mg/kg), % bare patch area (%BPA), and grain yield (t/ha) in 1990, Experiment 2. None was significant.

	Zn applied (kg/ha)	**Zn concentration (mg/kg)**	**% BPA**	**Grain yield (t/ha)**
Zn applied	1			
Zn concentration	0.675	1		
% BPA	-0.510	-0.491	1	
Grain yield	-0.058	0.305	-0.378	1

plant growth. When there was no effect of Zn on growth and yield, as occurred in 1990, there were also no significance in the Zn effects on disease severity. This is consistent with the view that the interaction between Zn and *Rhizoctonia* root rot severity is manifested over the Zn deficiency range only.

2.4.4. THEORETICAL IMPLICATIONS OF THE ZN-DISEASE INTERACTION.

The dependence of disease resistance on other limiting factors is now accepted by plant pathologists (van der Plank, 1975). The effect of nutrients on disease may be via growth, vigour and/or the biochemical pathways leading to resistance in the host plant, or through direct and indirect effects on the pathogen in the soil (Colhoun, 1973). In the case of root disease and nutrient stress, an interaction may be seen to arise in one of two ways: (i) the disease may damage the root system, thereby decreasing the nutrient absorbing surface, which ultimately leads to deficiency of the most limiting nutrient in the soil; or (ii) nutrient deficiency may adversely affect a biochemical pathway leading to resistance, resulting directly in an increase in disease severity (Graham, 1983). In the case of a Zn-*Fusarium* interaction, the evidence strongly points to the second of these two mechanisms (Sparrow and Graham, 1988).

The conclusions of Sparrow & Graham (1988) in a similar study with another root rot, together with the negative correlation from our study suggests that a high Zn concentration in plants may increase the resistance of cereals to *Rhizoctonia* root rot as well. This suggestion then becomes the hypothesis for the next series of studies in this thesis. The nature and mechanism of this interaction is clarified by experiments under controlled conditions that involve various levels of both Zn and inoculum, the first of which is reported in the next chapter.

CHAPTER 3

INTERACTION OF ZN AND INOCULUM LEVELS ON SEVERITY OF *RHIZOCTONIA* ROOT ROT OF WHEAT :

A preliminary pot experiment under controlled conditions

3.1. INTRODUCTION

In Chapter 2 it was found that the addition of Zn to a deficient soil suppressed the severity of *Rhizoctonia* root rot in cereals. This conclusion was based on the negative correlation between Zn and the severity of *Rhizoctonia* root rot in the field survey, as discussed in that chapter. To test the hypothesis that there is a causal relationship between Zn and disease, a factorial experiment involving different rates of Zn and inoculum treatments is required.

The technique developed by McDonald & Rovira (1985) seems to be successful in providing uniform inoculum of *R. solani* for pot experiments. The use as inoculum propagules of 1 mm diameter white millet seeds, sterile and colonized by the fungus, at the rates of 0, 8, 16 propagules per kg soil, provided a gradual increase of seminal root infection from 0 to 50 to 100%. The incubation period was also tested in the same study and a 2 week incubation before sowing was reported to be optimum to achieve maximum infection. Moreover, according to previous studies on nutrient-disease interactions done by Rovira *et al.* (1985), Wilhelm *et al.* (1988), and Sparrow & Graham (1988), at least 4 replications were needed to define the variation in this type of experiment. Their results experimentally explore various aspects of the epidemiology of this root disease. For rates of Zn application, Sparrow & Graham (1988) used three rates of 0, 0.06 and 12.0 mg Zn/kg soil to study the interaction of plane of Zn nutrition and crown rot of wheat caused by *Fusarium graminearum* Schw. Group 1.

However, since these techniques were used for a different disease and in different conditions, they needed testing for their suitability in our Zn-*Rhizoctonia* system. Therefore, in order to fine tune experimental conditions, and at the same time to examine in a preliminary way the possibility of a causal link in the correlation observed in the field, a simple pot experiment was conducted in a growth chamber with a combination of two Zn levels and three inoculum rates.

3.2. EXPERIMENT 3: MATERIALS AND METHODS

3.2.1. *Experimental design.* A factorial experiment combining two Zn levels (0 and 12 mg Zn/kg soil, designated Zn_0 and Zn_{12} respectively) and three inoculum rates (0, 8 and 16 propagules/kg soil; I_0, I_8 and I_{16}) was conducted in pots under controlled conditions. The experimental design was a Randomized Complete Block with five replicates.

3.2.2. *Inoculum.* Inoculum preparation was modified from the method of McDonald and Rovira (1985). White millet seeds, 1 mm in diameter, were soaked in reverse osmosis (RO) water overnight, drained, and autoclaved for 1 h at 121°C and 100 kPa in a 250 ml flask on two consecutive days. A pure culture of *Rhizoctonia solani* anastomosis group 8 was added as a 35 mm^3 cube taken from the edge of colonies actively growing on Czapek-Dox + yeast extract agar (Warcup, 1955). The inoculated millet was incubated at 25°C for 3 weeks (shaken weekly) to ensure complete colonization by the fungus. The millet was then air dried in a laminar flow cabinet.

3.2.3. *Soil.* A Zn-deficient siliceous soil (Laffer sand) from a naturally vegetated area at Tintinara in the southeast of South Australia, classified by Moore *et al.* (1983) as great group Quartzisamment according to USDA 1975 Taxonomy, was leached of soluble salts with RO water and air dried before use. Dry soil weighing 500g (560g wet soil at field capacity) was used for each pot. Polypropylene pots, 15 cm high and 7cm diameter, were used in this experiment.

3.2.4. *Basal nutrient.* A nutrient solution was prepared containing all nutrients, excluding Zn, using reverse osmosis water that had been passed through a Milli-Q® filtration system (Milli-Q® water). The basal nutrients (mg/pot) were: 117.6 NH_4NO_3, 61.8 K_2SO_4, 30.5 $MgSO_4.7H_2O$, 66.92 KH_2PO_4, 6.64 $MnSO_4.4H_2O$, 0.36 H_2MoO_4, 0.13 $CoSO_4.7H_2O$, 0.13 $NiSO_4.7H_2O$, 4.99 $CuSO_4.5H_2O$, 0.76 H_3BO_3, 6.64 $FeSO_4.7H_2O$ and 0.3% $CaCO_3$. The Zn solution was prepared from analytical grade $ZnSO_4.7H_2O$. $CaCO_3$ was mixed thoroughly with the dry soil before the solution was added to the soil.

3.2.5. *Seed.* Wheat seeds (*Triticum aestivum* cv Songlen), a Zn-inefficient variety which absorbs less Zn and produces less dry matter and grain yield in deficiency conditions (Graham *et al.*, 1992), 30-35 mg weight range, were surface sterilized in fresh 4% NaOCl for 2 min, rinsed with Milli-Q® water and germinated on moistened sterile filter paper for 36 h at 20°C before sowing.

3.2.6. *Procedure.* Dry soil (450 g) was thoroughly mixed with basal nutrient solutions and a Zn solution for the Zn_{12} treatment. For the inoculated treatments, 165 g wet soil was put into a pot, then, after spreading half of the inoculum onto the soil surface, another 165 g soil was added, followed by the rest of the inoculum and the final 165 g soil. For the uninoculated treatments, 495 g wet soil was simply put into the pot. This procedure was carried out with the treatment Zn_0 before the Zn_{12} treatment, in order to minimize Zn contamination. All pots were then watered to 85% field capacity, which is 10.2% moisture content (w/w), and incubated in a controlled environment chamber where the light was supplied by banks of high-intensity metal-halide lights which gave a light flux density of photosynthetically active radiation of 450 $\mu E\ m^{-2}\ s^{-1}$, set on a 10/14 h, 15 /10°C day/night cycle for 2 weeks before planting, to allow the fungus to colonize the soil. The temperatures and daylength chosen simulate conditions experienced in the field in southern Australia at the time of sowing.

After 2 weeks, three germinated seeds (as described in section 3.2.5) were placed on the soil surface of each pot followed by a further 50 g dry soil; water was added to make 12% moisture (w/w) content throughout. The soil surface was covered with acid-washed black plastic beads to a depth of 1 cm to reduce water loss and algal growth. The experiment was watered to field capacity regularly by weighing, left in the growth chamber under the conditions described above for 50 days and then harvested for plant height, top fresh and dry weight. Roots were scored for disease severity, as percent seminal roots infected, using the system of McDonald and Rovira (1985) and plant tops were analysed for Zn concentration by ICP, as previously described. All statistical analyses were performed using the GENSTAT 5 program (GENSTAT 5 Committee, 1989)

3.3. RESULTS

At 2 weeks after planting, the plants in the Zn_0 treatments were visibly smaller and less developed than those in the Zn_{12} treatment at every level of inoculum. Most of the plants showed symptoms of slight nitrogen deficiency and another 58.8 mg of NH_4NO_3 had to be applied at that stage to maintain normal growth. The classical symptoms of Zn deficiency were not visible until 30 days after planting, when the youngest fully emerged leaf blade (YEB) paled and developed a small necrotic spot that gradually extended from the middle of the leaf to the margins (Snowball and Robson, 1983). The plants in the I_{16} Zn_0 treatment were the first to show symptoms and were also the least vigorous (Plate 7).

Data for the number of tillers, height, top dry weight, root fresh weight, Zn concentration and Zn content in the plants at harvest are shown in Figure 3.1 (see Appendix 5 for ANOVA summary). Without Zn application, increasing inoculum density led to a significant reduction in tillering from 14 tillers/pot in the uninoculated treatments to 8 and 6 tillers/pot in the medium and high inoculum treatments respectively, whereas no significant difference in tillering was observed when Zn was applied. This

Plate 7 Songlen wheat plants, 50 days old, grown in Laffer sand with (three pots on right) and without (three pots on left) Zn application, Experiment 3. The inoculum treatments are 0, 8, and 16 propagules/kg soil from the left to the right respectively.

trend, especially the large difference between the inoculum treatments without Zn application, was also pronounced for root fresh weight and top dry weight. The plants in the I_0Zn_{12} treatment were smaller than expected because both top dry weight and root fresh weight were less than those in the corresponding treatment, I_0Zn_0. Roots of I_0Zn_{12} plants were thick and short although branching was normal (Plate 8). The visibly low yield of all the Zn_{12} treatments was probably due to Zn toxicity, since the Zn concentration in the plant tops in these treatments was as high as 1000 mg/kg.

The interaction between applied Zn and inoculum density was significant for growth, tillering and disease severity in terms of % seminal roots infected (%SRI) (Appendix 5). However, since there was no variance in disease severity of the uninoculated treatments (all zero %SRI), another ANOVA was calculated without this treatment to examine the difference between the remaining two inoculated treatments (Appendix 6); the effect of Zn was confirmed by the significant difference in disease scores between Zn treatments. Percent seminal roots affected decreased from 98% and 100% in the Zn_0 treatments to 10% and 25% in the Zn_{12} (Figure 3.2).

3.4. DISCUSSION

3.4.1. SUPPRESSION OF DISEASE BY ZN.

The lower disease scores (percentage of seminal roots infected or %SRI) by *R. solani* in the Zn_{12} treatments of these experiments compared to the Zn_0 show that Zn can spectacularly suppress disease severity under these conditions. This is the first time that the Zn-*Rhizoctonia* interaction has been established systematically in a factorial experiment and the result supports results from the field and a much earlier observation of Millikan (1938). Hall (1984) failed to show an effect of Mn on severity of *R. solani* in a similar type of study, which suggests a specificity in the effects of Zn on this pathogen, a conclusion which is supported by the correlation coefficients of Table 2.1. P. Wall

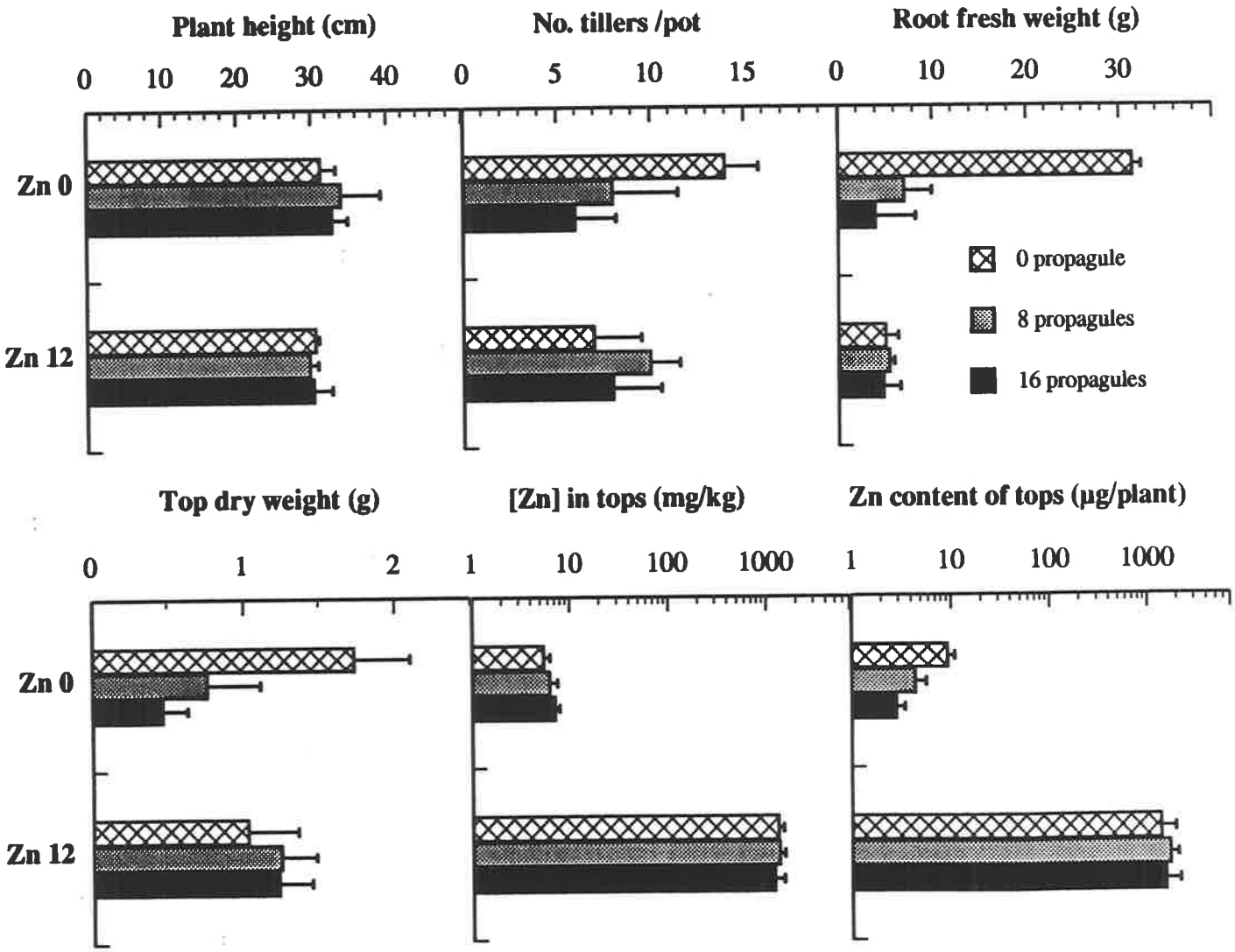


Figure 3.1 Growth of 50 day old Songlen wheat plants, in terms of number of tillers, height, top dry weight, root fresh weight, Zn concentration and Zn content in plant tops. Experiment 3. Error bars show standard errors .

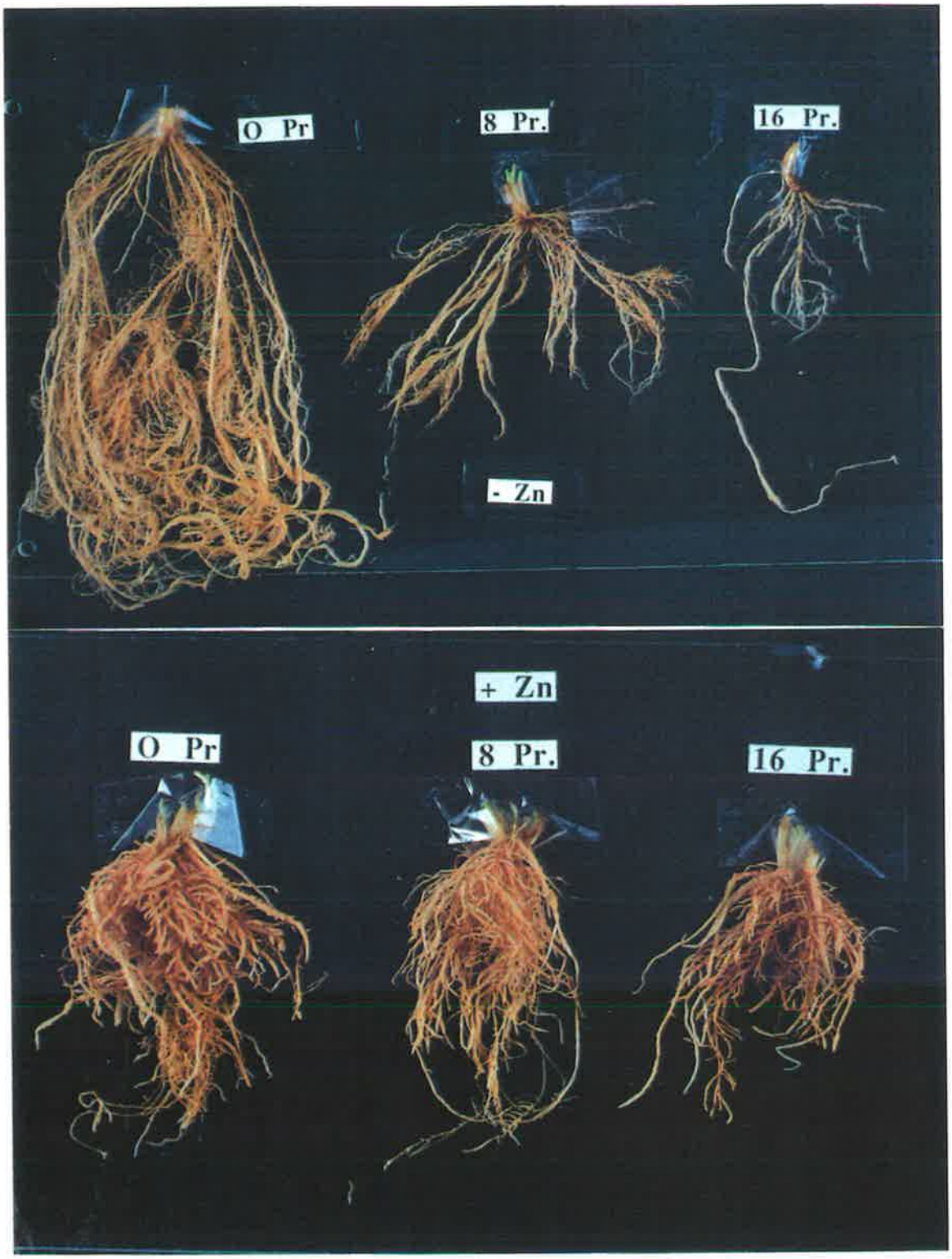


Plate 8 Roots of Songlen wheat plants, 50 days old, grown in Laffer sand without (above) and with (below) Zn application, Experiment 3. The inoculum treatments are 0, 8, and 16 propagules/kg soil from the left to the right, respectively. Note the abnormal root growth indicative of a toxic effect of Zn.

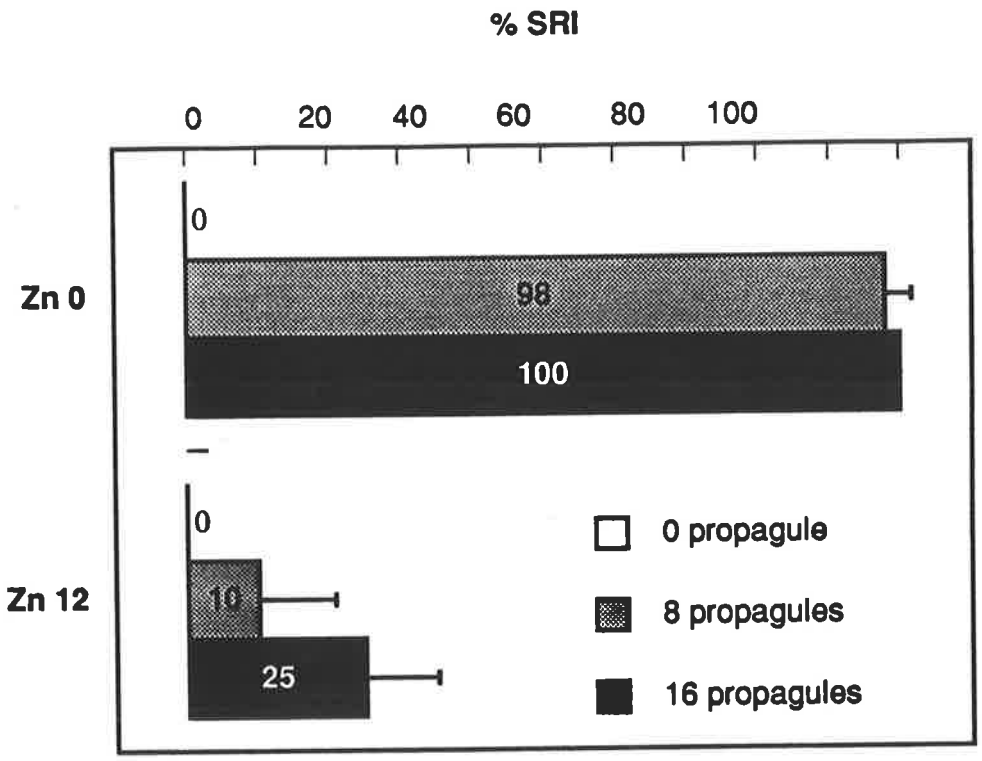


Figure 3.2. Disease severity measured as % seminal roots infected (%SRI) of 50 days old wheat plants with and without Zn application at three levels of added inoculum. Experiment 3. Error bars show standard errors of means over five replications.

(personal communication) observed an interaction between disease severity and nitrogen application but the variation in severity was too high for significance in the analysis of variance and he had to demonstrate the relationship by multiple regression.

3.4.2. A CAUSAL LINK BETWEEN ZINC CONCENTRATION AND *RHIZOCTONIA* ROOT ROT SEVERITY.

As previously discussed in Chapter 2, the relationship between root disease and nutrient stress could be considered to arise in one of two ways. One possibility is that disease is the cause of nutrient deficiency by pruning the root system and thus decreasing the mineral uptake; if so, in this case, increasing disease severity should have resulted in reduced Zn concentration in the plants. Alternatively, nutrient deficiency, by its adverse effect on physiological processes, predisposes the plant to the disease.

Results from this experiment (Figure 3.2.) do not support the first argument. There is no statistical difference in Zn concentration between the 3 inoculum levels within the same Zn application rate so the low Zn concentration due to the putative root pruning effect caused by inoculum treatments cannot be confirmed. On the other hand, there were pronounced differences in severity between Zn treatments. From this result, it may be concluded that increasing disease severity through increased inoculum density did not induce Zn deficiency in the plants by reducing the Zn concentration in the plant tissue. In contrast, it demonstrated that increasing Zn concentrations in the plant tissue resulting from Zn application obviously did reduce disease severity in both the medium and high inoculum density treatments. Thus, the result established that the disease did not enhance Zn deficiency, but rather, that Zn deficiency enhanced the host plants susceptibility to disease. Therefore, the alternate hypothesis that Zn deficiency is the cause of increasing predisposition to disease is preferred.

3.4.3. LIMITATIONS OF THE EXPERIMENTAL TECHNIQUES.

3.4.3.1. The imbalance of basal nutrients and level of Zn application.

According to growth observations and tissue chemical analysis (Appendix 4), it appeared that the plants received a slightly lower level of nitrogen, and higher levels of phosphorus, potassium, manganese and boron than desirable. This nutrient imbalance may explain why the Zn deficiency symptoms in the nil Zn treatments developed later than expected. These data were used to adjust the balance of the basal nutrients and Zn in the next experiment.

According to Godbold *et al* (1983), abnormal root growth is a Zn toxicity symptom. The very high concentration of Zn found in the plant tissue tended to support this speculation. The level of Zn applied in this treatment was reported to be appropriate by Sparrow & Graham (1988) in their study on Zn-*Fusaruium* crown rot interaction, which used bigger pots and a different inoculation procedure. The very high dosage of Zn in this experiment also made it hard to conclude whether decreased infection was because of a positive effect of Zn addition on plant resistance, or a suppressive effect of Zn on the fungus in the soil. Suitable Zn levels for the study of the Zn-*Rhizoctonia* root rot system need to be established in further studies.

3.4.3.2. The inoculum rates and root scoring system.

In studies of this kind, the use of inoculum as a treatment factor was developed by Graham & Rovira (1984) and Rovira *et al.* (1985) who used finely ground oat seed infected with *Gaeumannomyces graminis* var. *tritici (Ggt)* as inoculum in their pot studies of the interaction between take-all disease and manganese in wheat. Wilhelm *et al.* (1988) successfully used an agar plug of *Ggt* inoculum for their pot experiment with very small containers, whereas Hall (1984) did not get satisfactory results from the use of an agar plug inoculum with *R. solani.* Because the natural inoculum of this fungus in the field is mycelium-colonized organic debris, there have been problems in uniformity of

propagule size, virulence and age of inoculum that make it difficult to relate results from the pot experiments to field conditions. Dubé (1971) proposed the technique of growing plants in an undisturbed soil sample collected from the patch itself, a technique which appeared to be quite useful, but propagule density still could not be quantified. The use of fungus-colonized millet seeds suggested by McDonald & Rovira (1985) seems to be a better solution to quantifying propagule density in the pot experiment, providing a more gradual increase in the disease severity between these two inoculum rates in their experiment, which was in a different soil. However, results from the experiment in this chapter suggested that this propagule might not be suitable for the study of Zn-*Rhizoctonia* interaction because disease severity, in terms of %SRI, was not much different between the treatments of 8 propagules/kg soil (98%SRI)and 16 propagules/kg soil (100%SRI). This aspect needs further investigation.

The root scoring system for disease severity based on the %SRI has also been suspected of being inappropriate for pot experimentation. Although most of the seminal roots in the 8 propagule treatment were infected, these roots were still longer than those in the 16 propagules treatments and, therefore, presumably able to absorb and provide more water and nutrients to the plant. This disease scoring system seemed to be suitable for the plants sampled from the field in the previous study as considerable variation in %SRI among plants was detected. However, in a pot study, the scoring system may be less discriminating because of the limited volume of soil in the pot and high root density that occurs. In this case, measures of the performance of the root, such as root length, may have to be taken into account.

To overcome all of these limitations, more suitable techniques for the study of interactions in the Zn-*Rhizoctonia* root rot system needed to be established, and these are the subject of the next chapter.

CHAPTER 4

ESTABLISHING TECHNIQUES FOR THE SUBSEQUENT POT STUDY

4.1. INTRODUCTION

Results from the previous chapter strongly suggest that Zn deficiency in wheat plants aggravates the severity of *Rhizoctonia* root rot. However, some limitations in the techniques, such as the level of Zn and basal nutrients used, inoculum levels and the disease scoring system, made it difficult to precisely identify the nature and mechanism of the Zn effects on disease.

Experiments in this chapter describe the search for the optimum nutrient levels and inoculum techniques to be used for a major pot experiment to study the Zn-*Rhizoctonia* root rot interaction in detail.

4.2. RANGE OF ZN APPLICATIONS FOR THE POT EXPERIMENT

The experiments in this section were aimed at observing the relation of wheat growth to Zn nutritional status at different levels of Zn application to Laffer sand in pots, in order to assess the proper rates of Zn for further study.

4.2.1. EXPERIMENT 4.1

4.2.1.1. Materials and Methods

4.2.1.1.1. *Design.* A Randomized Complete Block design was used, with three replications and six Zn treatments. These are:

i) 0 mg Zn/kg soil without $CaCO_3$

ii) 0 mg Zn/kg soil plus 0.3% $CaCO_3$

iii) 0.002 mg Zn/kg soil plus 0.3% $CaCO_3$

iv) 0.004 mg Zn/kg soil plus 0.3% $CaCO_3$

v) 0.020 mg Zn/kg soil plus 0.3% $CaCO_3$

vi) 12.0 mg Zn/kg soil plus 0.3% $CaCO_3$.

To confirm the toxic effect of Zn in Experiment 3 in Chapter 3, the lowest and the highest rates of Zn were the same as in that experiment, with more rates of Zn in between to give a better distribution of points from the deficient to the adequate range.

4.2.1.1.2. *Soil.* A Zn-deficient Laffer sand was used, leached of soluble salts with reverse osmosis water and air dried, as described in Chapter 3.

4.2.1.1.3. *Basal nutrients.* A nutrient solution, as used in Chapter 3, was prepared containing all essential nutrients, excluding Zn, using reverse osmosis water that had been passed through a Milli-Q® filtration system (Milli-Q® water). The basal nutrients (mg/pot) were: 117.6 NH_4NO_3, 61.8 K_2SO_4, 30.5 $MgSO_4.7H_2O$, 66.92 KH_2PO_4, 6.64 $MnSO_4.4H_2O$, 0.36 H_2MoO_4, 0.13 $CoSO_4.7H_2O$, 0.13 $NiSO_4.7H_2O$, 4.99 $CuSO_4.5H_2O$, 0.76 H_3BO_3, 6.64 $FeSO_4.7H_2O$; and $CaCO_3$ 0.3% (w/w). The Zn solution was prepared from analytical grade $ZnSO_4.7H_2O$ according to the required concentration previously mentioned in 4.2.1.1.1.

4.2.1.1.4. *Seed.* Seeds of Songlen wheat similar to the one in Chapter 3 were used. They were surface sterilized and pregerminated as described before.

4.2.1.1.5. *Procedure.* Dry soil for each pot was weighed in a plastic bag, mixed thoroughly with $CaCO_3$, followed by basal nutrients and Zn solution according to treatment, and then put into a polypropylene pot 15cm high and 7cm in diameter. In each pot, three pre-germinated seeds (as in 4.2.1.1.4) were sown at 1 cm depth. Afterwards, the pots were mulched with acid-washed black plastic beads to a depth of 1 cm to reduce water loss and algal growth, watered to 12% moisture content and then kept in a growth chamber under a light flux density of photosynthetically active radiation of 450 $\mu E\ m^{-2}\ s^{-1}$, set on a 10/14 h, 15 /10°C day/night cycle, simulating winter

conditions in the field. The experiment was watered to field capacity regularly until Zn deficiency symptoms appeared. At 50 days after sowing, the plants were harvested for top and root fresh and dry weight, and analysed for Zn in whole shoots by ICP, as previously described. All of the measurements were statistically analysed with the appropriate procedure.

4.2.1.2. Results

At about 30 days after planting, plants in Experiment 4.1 became N deficient without any symptoms of Zn deficiency, as in Experiment 3. Another 58.8 mg of NH_4NO_3 was added and then 5 days later, Zn deficiency symptoms started to appear in the plants of the Zn_0 treatment followed by those of $Zn_{0.002}$ and $Zn_{0.004}$. No symptoms appeared in the $Zn_{0.02}$ and Zn_{12} treatments by the time of harvesting. Growth in terms of top fresh and dry weight, root fresh and dry weight, and number of Zn deficient leaves per pot in Experiment 4.1 are shown in Table 4.1. Without Zn application, plants in the pots with $CaCO_3$ tended to have better top and root growth, but more severe Zn deficiency according to the number of Zn deficient leaves, than plants in the pots without $CaCO_3$. In the $Zn_{0.02}$ treatment, there were no symptoms of Zn deficiency on the leaves, even though the top growth was not significantly different from that of the next lowest Zn treatment, $Zn_{0.004}$, which had approximately 8 Zn-deficient leaves. Generally, there were no statistical differences between Zn applications for growth of the wheat plants, except for the Zn_{12} treatment where the top fresh and dry weights were significantly higher than for the other treatments in which lower rates of Zn were applied. There were slight declines in root fresh and dry weights in the Zn_{12} compared to the other lower Zn treatments, but the differences were not statistically significant. Zn concentration in relation to the dry weight of tops, (response curve shown in Figure 4.1), indicated that most of the plants, except in the Zn_{12} treatment, had Zn concentrations in their tops which were lower than 20 mg/kg, that is, in the range of Zn deficiency. Zn concentrations in the shoots of the plants in the Zn_{12} treatment were around 1000 mg/kg, which is considered to be in the toxic range (Reuter & Robinson,

1986).

4.2.1.3. Discussion

In this experiment, the plants showed symptoms of inadequate N, and this explains why the Zn deficiency symptoms appeared so late in the growing period. The response of plants to Zn application depends on the N status of the plants. According to Al-Samerria (1984), N deficient plants did not respond to applied Zn, whereas plants with an adequate level of N responded strongly to Zn application. Zn deficiency was induced in plants grown in low Zn soil at high applied N by increasing plant growth to the point where the absorbed Zn was diluted within the plant to a deficient concentration. Moreover, ICP analysis of all elements (Appendix 4) also showed slightly low levels in K, P and Mg, and slightly high levels of Fe and Mn. This trend suggests that other nutrient imbalances may have delayed the effects of Zn deficiency in this experiment, and also the experiment in Chapter 3. Most of the Zn levels used in this experiment were also in the Zn deficient range except the highest level that seemed to be toxic. These levels were adapted from an experiment of Sparrow & Graham (1988) in which bigger pots and a different wheat variety were used.

This result suggested that the rates of Zn and amount of basal nutrients, especially N, should be adjusted which is the purpose of Experiment 4.2.

4.2.2. EXPERIMENT 4.2

4.2.2.1. Materials and Methods

In this experiment, the design and most of the materials and methods were similar to Experiment 4.1, but there were some alterations based on the analysis of the plants in Experiment 4.1. Firstly, rates of Zn were 0, 0.01, 0.04, 0.1, 0.4 and 6.0 mg Zn/kg soil, with an application of 0.3% $CaCO_3$ in every treatment. Thus, the low rates

Table 4.1 Growth of Songlen wheat, 50 days old, grown at increasing levels of applied Zn in a Zn-deficient Laffer sand, Experiment 4.1. Means followed by the same letter are not significantly different according to the Duncan Multiple Range Test.

Zn applied (mg/kg soil)	Top Dry wt. (g)	Top fresh wt. (g)	Root Dry wt. (g)	Root fresh wt. (g)	Zn deficient leaves/pot
0 -Ca	1.09 b	7.92 c	0.925 b	23.62 b	12.7 bc
0 +Ca	1.11 b	8.20 bc	2.145 a	29.44 a	13.7 c
0.002+Ca	1.13 b	8.15 bc	1.681 ab	28.97 ab	10.7 bc
0.004+Ca	1.26 b	8.48 bc	2.170 a	29.59 a	7.7 b
0.02 +Ca	1.31 b	8.72 b	2.163 a	30.31 a	0 a
12.0 +Ca	1.65 a	9.46 a	1.238 ab	27.84 ab	0 a

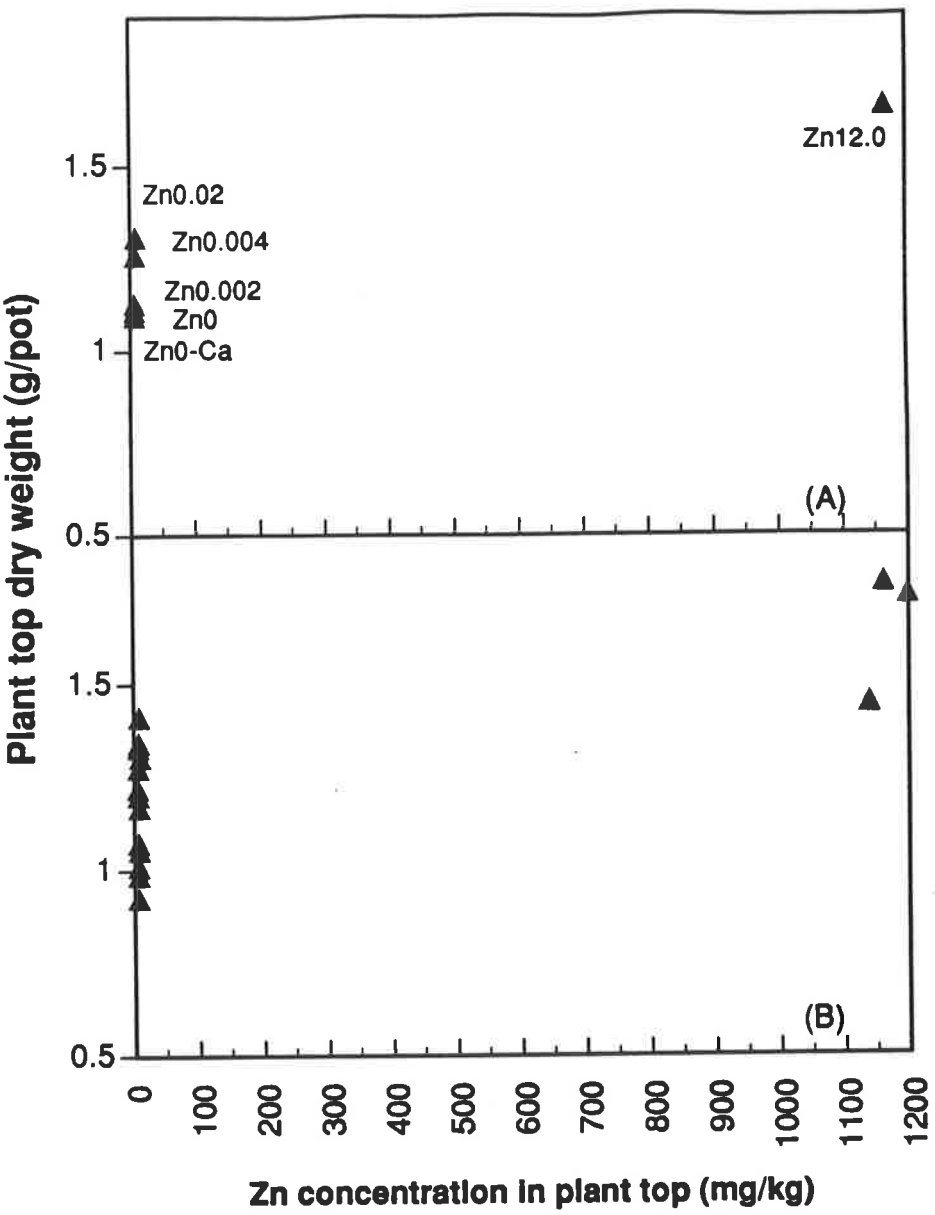


Figure 4.1 Response in yield of dry matter of Songlen wheat at 6 levels of applied Zn as a function of Zn concentration in the youngest fully emerged leaf blade (YEB), Experiment 4.1. Each data point in (A) represents the average of three replicates, and in (B) the individual replicates are shown.

were increased and the high rate decreased. Secondly, the following basal nutrients (mg/pot) were adjusted to be: 176.4 NH_4NO_3, 45.75 $MgSO_4.7H_2O$, 3.32 $MnSO_4.4H_2O$, 0.18 H_2MoO_4, 0.07 $CoSO_4.7H_2O$, 0.07 $NiSO_4.7H_2O$, 1.13 H_3BO_3 and 0.33 $FeSO_4.7H_2O$; the others remained as in Experiment 4.1.

The experiment was harvested at 21 days after planting and the same parameters were measured as in Experiment 4.1.

4.2.2.2. Results

Figure 4.2 shows plant growth measured as height, top dry weight and root dry weight from Experiment 4.2. All of the growth measurements were lowest in the Zn_0 treatment, followed by the treatments of $Zn_{0.01}$ and $Zn_{0.04}$, but with no considerable differences. For plant height and top dry weight, there were distinct increases when Zn application was increased from $Zn_{0.04}$, where the height was around 22 cm with top dry matter around 0.12 g/pot, to $Zn_{0.1}$, where the plants were 30 cm high and around 0.28 g/pot in dry matter. This level of growth seemed to be maintained at the remaining two higher levels of applied Zn. There was a trend of increasing root growth according to the increasing Zn applied but the variation is too high to conclude that any significant differences existed between adjacent means. The relationship of top dry weight and Zn concentration in the tissue (Figure 4.3) shows a big jump in top dry weight from 0.16 g/pot with around 15 mg Zn/kg in the tissue at Zn0.04 treatment to around 0.28 g/pot with Zn concentration of 25 mg Zn/kg in tops from the Zn0.1 and Zn0.4 treatments. Although the Zn concentration in plant tops reached a maximum of 150 mg/kg in the Zn6.0 treatment, top dry weight remained at a level of 0.28 g/pot which was, therefore, considered to be the yield plateau.

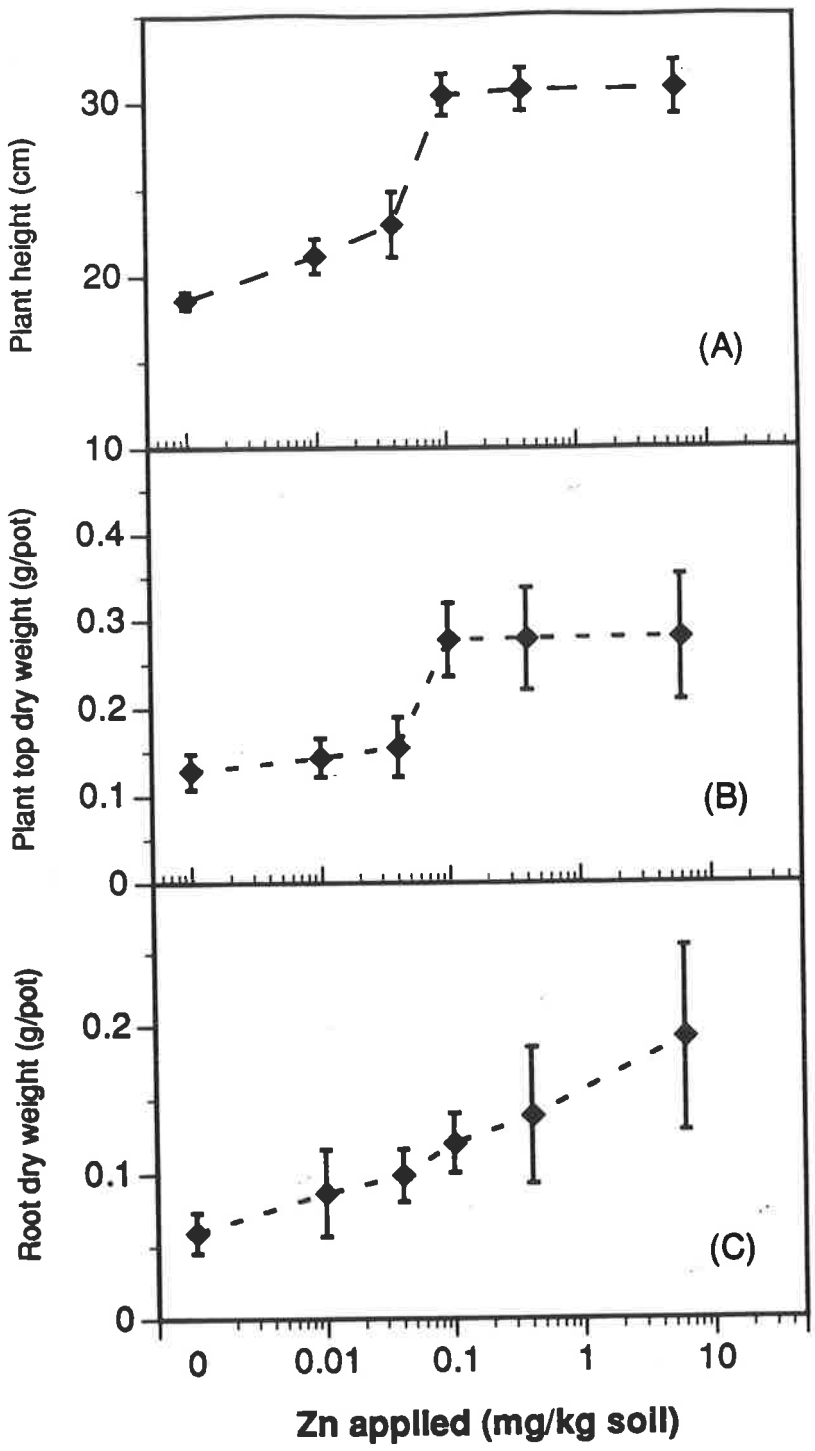


Figure 4.2 Growth of 21 day old Songlen wheat grown in pots supplied with Zn at six different rates in a Zn-deficient Laffer sand, in terms of (A) height, (B) top dry weight and (C) root dry weight. Experiment 4.2.

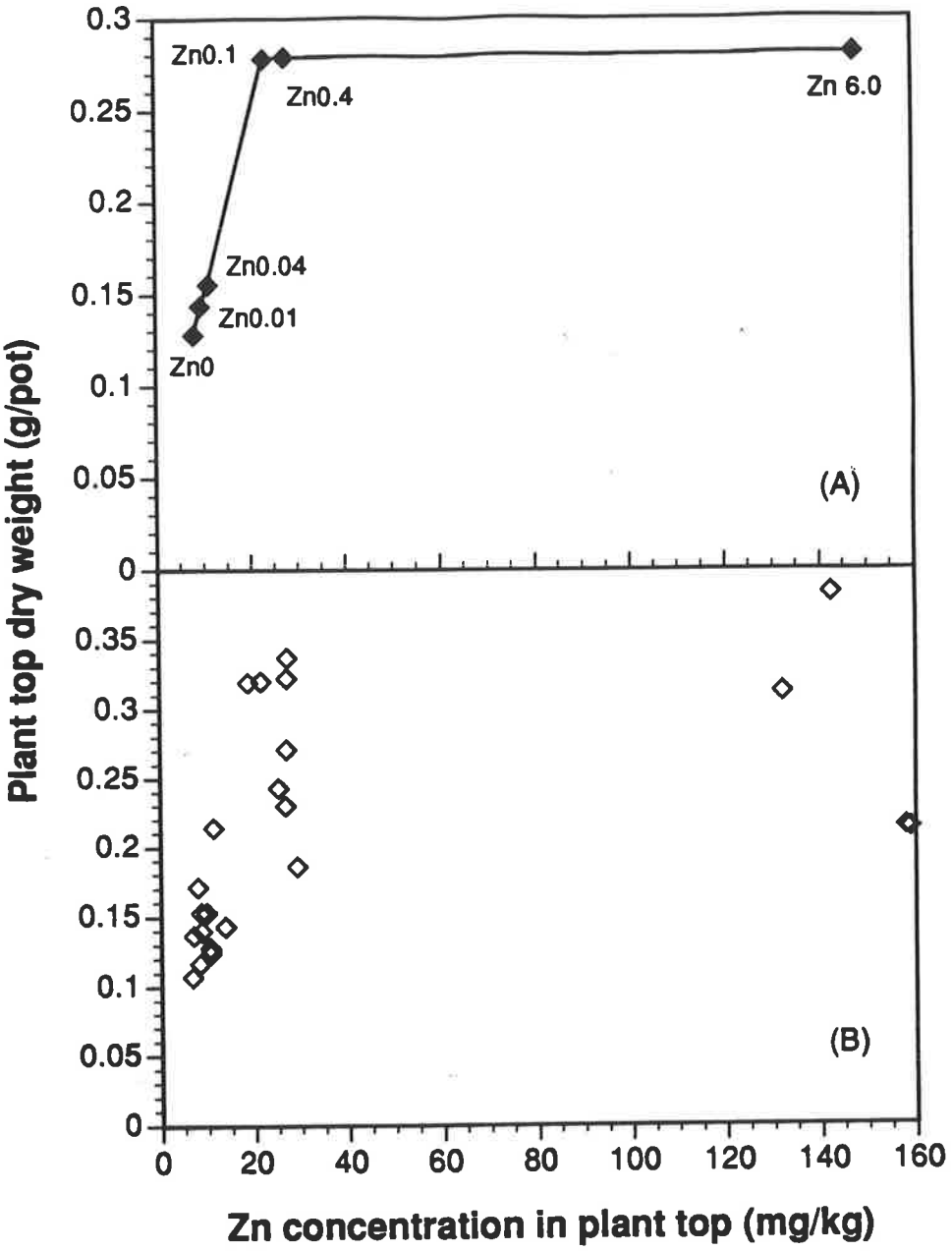


Figure 4.3 Top dry weight of Songlen wheat grown with 6 levels of applied Zn as a function of Zn concentration in tissue, Experiment 4.2. Each data point in (A) represents the average of four replicates and in (B) individual replicates are shown. The data show the Zn critical concentration is in the range of 15-25 mg/kg, which occurs between treatments of Zn 0.04 and Zn 0.1.

4.2.2.3. Discussion

The amounts of nutrients used in this experiment were adjusted from Experiment 4.1, and the new rates seemed to be more appropriate, as Zn deficiency symptoms appeared within 21 days of planting, much sooner than in previous experiments. The Zn levels in this experiment were also distributed over a narrower range from deficiency to adequacy, and seemed to be more appropriate to our system in the experiments to follow.

The results also suggested that the transition stage from Zn deficiency to Zn adequacy might fall between the application levels of 0.04 and 0.1 mg Zn/kg soil, and 0.1 mg Zn appears to be the lowest rate of applied Zn required to reach maximum growth under these conditions. Furthermore, although this experiment was not designed to define the critical concentration for Zn in wheat, it can be seen that the critical concentration of Zn in whole tops lies between 15-25 mg/kg. This is in substantial agreement with those values reported by Reuter & Robinson (1986) and Marschner (1989).

4.2.3. Conclusion

The rates of applied Zn of 0, 0.01, 0.04, 0.1, 0.4 and 6.0 mg Zn/kg soil and the basal nutrients, as used in Experiment 4.2, are more appropriate for the pot system used to study the interaction of Zn-*Rhizoctonia* root rot in the following chapter.

4.3. EFFECT OF SOURCE AND DENSITY OF INOCULUM OF *R. SOLANI* ON THE DISEASE SEVERITY OF WHEAT IN SMALL POTS

The most important source of natural inoculum for *R. solani* has been found to be chains or bundles of hyphae colonising organic debris in the soil (Neate, 1987). To establish a pot experiment, a uniform size and virulence of propagule is desirable. Henis

& Ben-Yephet (1970) prepared propagules for R. *solani* causing damping off in bean by using a blender to homogenize the PDA agar with the growing fungus and demonstrated that only propagules larger than 250 µm added to natural soil could infect bean seedlings. It was also difficult to get uniform size and amounts of fungus from their method. Wilhelm (1991) succeeded in using a uniform size of agar disc cut from the edge of actively growing colonies of *Ggt* as an effective inoculum for studies of the take-all disease, but Hall (1984) used the agar disc inoculum for *R. solani* with unsatisfactory results. Moreover, Kamal & Weinhold (1967) suggested that *R. solani* will not function as a pathogen without a supply of plant organic matter. McDonald & Rovira (1985) overcame the problem of non-uniform propagules of *R. solani* by using uniformly sized millet seed colonised by the fungus, and found this method was successful in their study. However, according to the system used in Chapter 3, the results (Figure 3.2) showed that there was not much difference in terms of %SRI between the low and high inoculum levels. Dr S M Neate & A Banger (unpublished data), compared millet seed and wheat bran inocula at the same diameter and range of inoculum rates, and found that uniformly sized wheat bran provided levels of disease severity which varied according to the inoculum rate more than the millet seed did.

To test a suitable food-base for inoculum to be used in the Zn-*Rhizoctonia* system, a factorial experiment was conducted in the growth chamber to compare disease severity from 2 types of inocula, wheat bran (WB) and millet seed (MS), without Zn application.

4.3.1. MATERIALS AND METHODS

4.3.1.1. *Experimental design.* The experiment was a 2x4 factorial design in 3 replications, with the treatment combinations being 2 types of inocula (wheat bran and millet seed) and 4 inoculum densities: 0, 2, 4 and 8 propagules/kg soil, i.e. 0, 1, 2 and 4 propagules/pot.

4.3.1.2. *Inoculum.* Wheat bran and millet seed inocula were prepared from the method of McDonald & Rovira (1985) modified as described in Chapter 3, as follows. The uniformly sized white millet seeds and wheat bran (1 mm diameter) were soaked separately in RO water overnight, drained, and autoclaved at 121°C and 100 kPa in a 250 ml flask on two consecutive days for 1 h. Pure culture of *R. solani* anastomosis group 8 was added as a cube of 35 mm^3 taken from the edge of colonies actively growing on Czapek-Dox + yeast extract agar medium (Warcup, 1955). The inoculated millet and wheat bran were incubated at 25°C for 3 weeks (shaken weekly) after which time they were air dried in a laminar flow cabinet.

4.3.1.3. *Soil & Pot.* Laffer sand, a Zn-deficient siliceous soil, as used in Experiments 3, 4.1 & 4.2 was used after leaching using the procedure previously described. Dry soil weighing 500 g (the equivalent of 560 g wet soil at field capacity) was used for each polypropylene pot, 15 cm high and 7 cm in diameter, as in the others.

4.3.1.4. *Basal nutrients.* A nutrient solution was prepared using Milli-Q® water, which contained all of the essential nutrients, excluding Zn. The nutrient concentrations were adjusted according to analysis of plant tissue to be similar to the one in Experiment 4.2 which gave a better balance of nutrients. The basal nutrients (mg/pot) were: 176.4 NH_4NO_3, 61.8 K_2SO_4, 45.75 $MgSO_4.7H_2O$, 66.92 KH_2PO_4, 3.32 $MnSO_4.4H_2O$, 0.18 H_2MoO_4, 0.07 $CoSO_4.7H_2O$, 0.07 $NiSO_4.7H_2O$, 4.99 $CuSO_4.5H_2O$, 0.76 H_3BO_3, 0.33 $FeSO_4.7H_2O$

4.3.1.5. *Seed.* Wheat seeds of a Zn-inefficient variety, Songlen, was used as in the previous experiments.

4.3.1.6. *Procedure.* Dry soil (450 g/pot) was thoroughly mixed with 0.3% $CaCO_3$ and basal nutrient solution. The inoculum treatments were created as described in Experiment 3. All pots were then watered to 10.2% moisture content (w/w), which is 85% field capacity, and incubated to allow the fungus to colonize the soil for 2 weeks in a controlled environment with similar conditions to those described in Chapter 3, which

simulated conditions experienced in the field in southern Australia at the time of sowing.

After 2 weeks, three germinated seeds were placed on the soil surface of each pot followed by a further 50 g dry soil; water was added to make the whole pot up to 12% soil moisture content (w/w). The soil surface was covered with acid-washed black plastic beads to a depth of 1 cm to reduce water loss and algal growth. The experiment was watered to field capacity regularly, left in the growth chamber under the conditions described above for 30 days and then the plants were harvested, and measurements made for plant height and top fresh and dry weight.

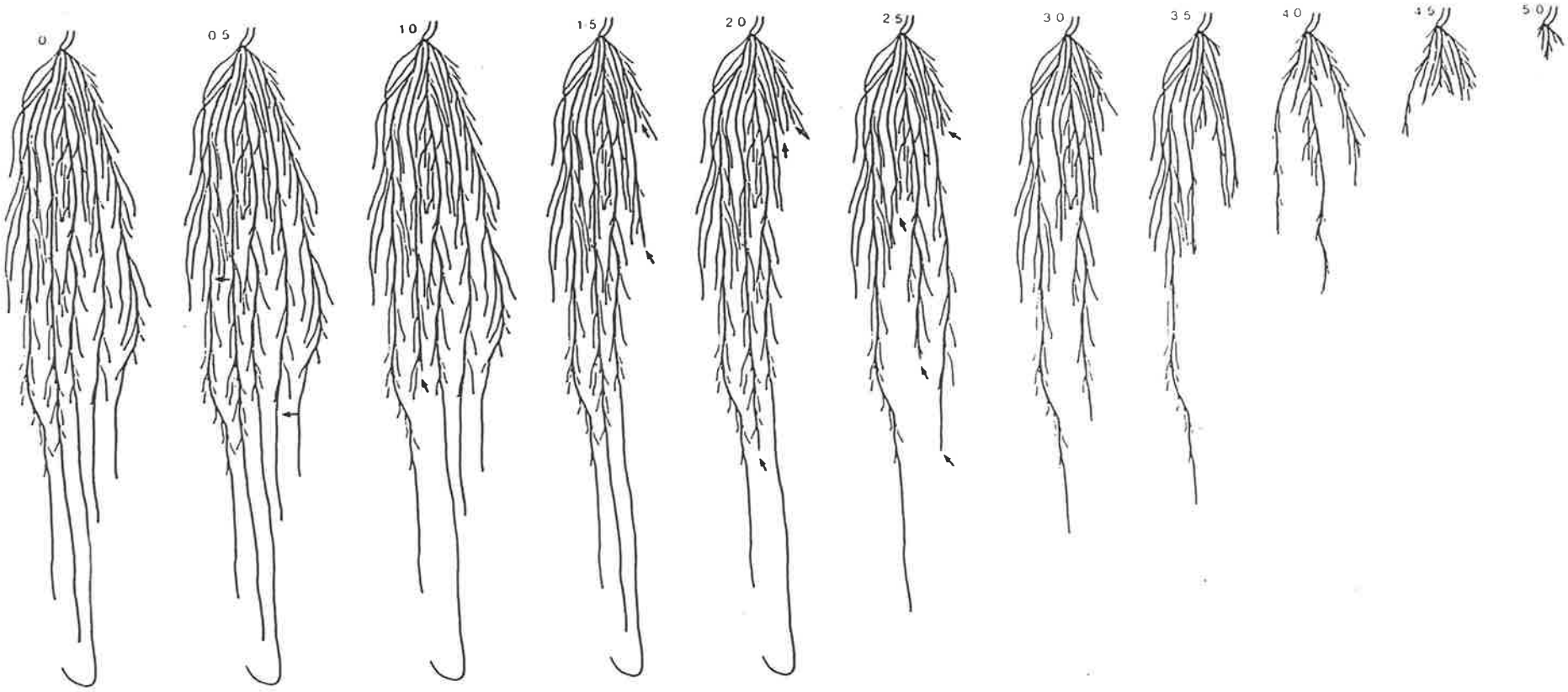
Finally, plant tops were analysed for Zn concentration by ICP, as previously described. All of the measurements were statistically analysed with the appropriate ANOVA for the factorial design.

Roots were scored for disease severity in 2 ways. Firstly, the axial length of all the seminal roots in each plant was measured and an average seminal root length for each treatment was calculated. Secondly, the root systems of the plants from all treatments were also assessed visually by reference to Figure 4.4, using the root rot scale developed by Dr G C MacNish based on the performance of the root (Sweetingham and MacNish, 1992). The disease score steps from 0.5 to 5.0 in 0.5 increments and is inversely related to the root length of seminal axes. Scores of 0 and 0.5 have the same length of root, but 0.5 has clear evidence of lesions. These two scoring systems were then correlated.

4.3.2. RESULTS

The relationship between seminal root length and disease score is shown in Figure 4.5. The seminal root length ranged from 22 cm in uninfected root systems to as low as 3 cm in the highest rating of around 4. The linear correlation is highly significant with $R^2 = 0.96^{**}$

Figure 4.4 Root rot scale for disease scoring system developed by Dr. G C MacNish (Sweetingham & MacNish, 1992) at half actual size. The disease score steps from 0.5 to 5 in 0.5 increments, inversely related to the root length of seminal axes. Arrows indicate points of spear tips or truncated roots.



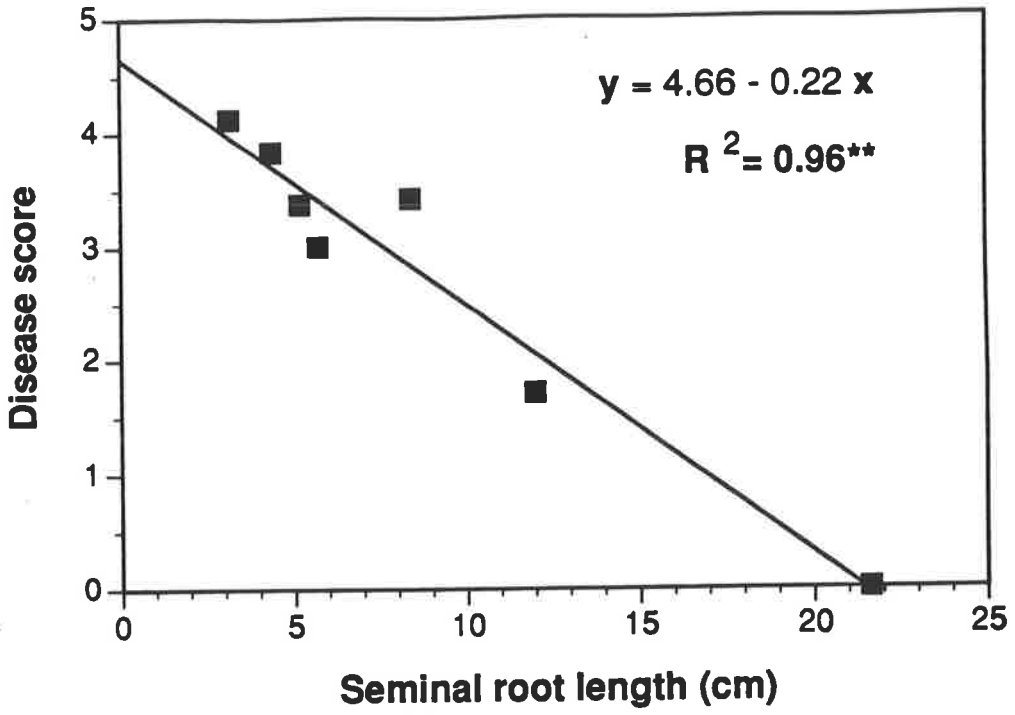


Figure 4.5 Linear correlation between seminal root length and disease score measured according to Figure 4.4 (Sweetingham & MacNish,1992). The relationship is highly significant.

Disease score, plant height, plant top dry weight and root dry weight are shown in Figure 4.6. Disease scores of roots from the plants inoculated with wheat bran inoculum were distributed from 1.5 to 3 and 3.5 in the 1, 2 and 4 propagules/pot respectively, while in the millet inoculum treatments, the disease scores were 3.3, 3.8 and 4.2 for 1, 2, and 4 propagules/pot treatments respectively. All of the growth indices also decreased when disease severity increased according to the inoculum rates, but the growth reduction in wheat bran inoculum treatments was less than in the millet seed ones.

4.3.3. DISCUSSION

R. solani is considered to be one of the 'translocating fungi' which are able to translocate nutrients from a food base through an established mycelium via protoplasmic streaming in the hyphae (Schütte, 1956). Such fungi, instead of passively waiting in the form of resting propagules or spores for a root or substrate to approach them, as do the 'non-translocating fungi', grow actively through the soil as an expanding, subspherical colony of mycelium from a colonized substrate towards living roots or to plant residues in the soil as a new substrate (Garrett, 1970). This might explain why cutting agar discs from the growing margins of a colony as used by Hall (1984) had not worked well for pot experiments.

Results from this experiment demonstrate that the wheat bran gave a wider range of disease severity than did the millet, although mycelia from both types of propagule grew out equally fast on agar plates. The difference in disease severity might be due to the size of the food base in the propagule as the average dry weight of a millet seed propagule was 5 mg, much larger than a wheat bran propagule which weighed around 1 mg. The more distributed range of disease scores from wheat bran inoculum was also desirable as nutritional effects of the type to be studied in the following experiments tend to be suppressed by exceptionally high levels of disease (Graham & Webb, 1991).

Both disease assessment methods seem to be valid representatives of disease severity, as the root performance and top growth for each treatment decreased according

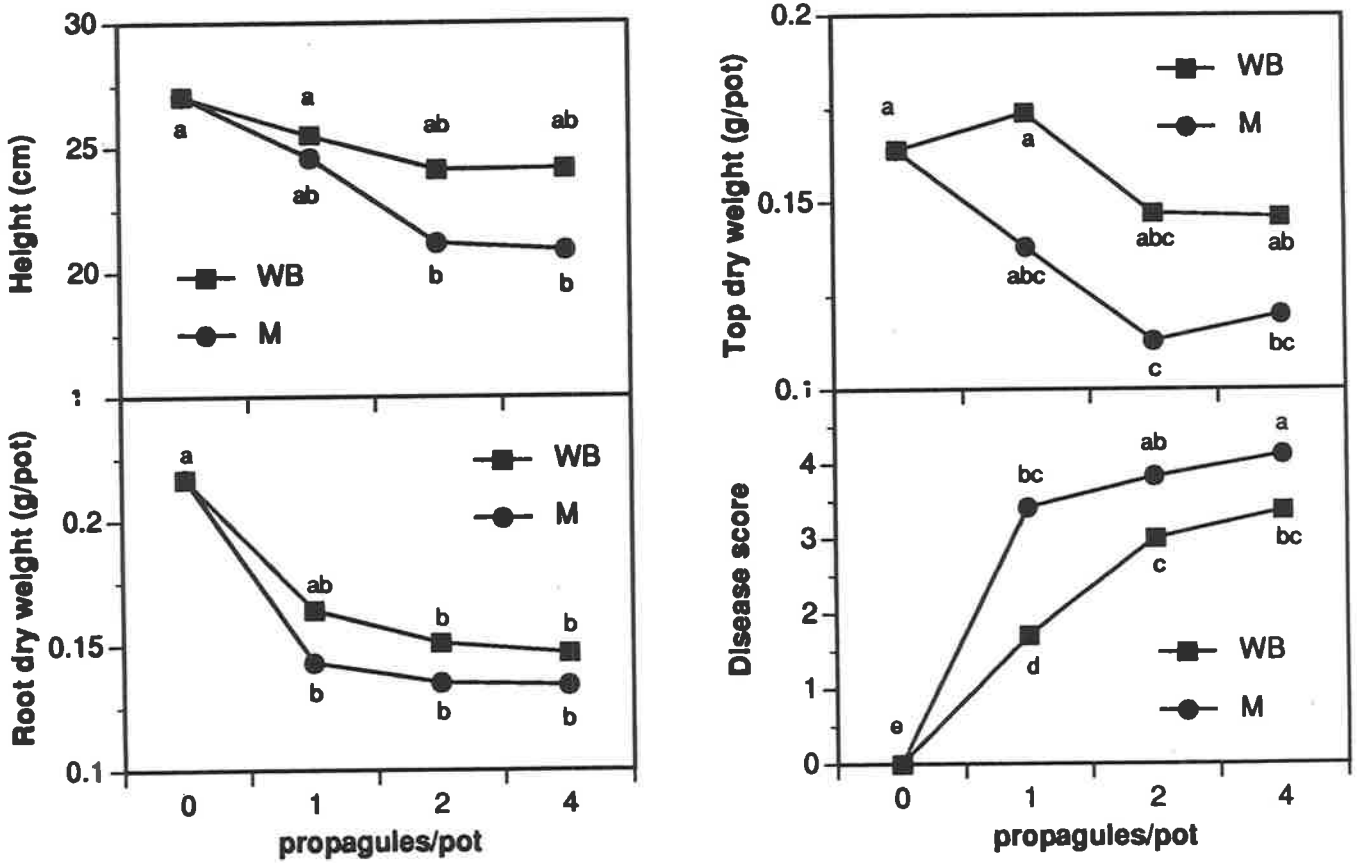


Figure 4.6 Plant growth in terms of height, top dry weight, root dry weight and disease severity of Songlen wheat grown in pots of Zn-deficient Laffer sand inoculated with *R. solani* via millet seed (M) or wheat bran (WB) propagules. Experiment 4.3.

to the increase of both seminal root length and disease score. However, since scoring is quicker and more convenient than measuring root length and the indices were significantly correlated, the disease score method seems to be preferable.

So, in conclusion, it was decided that, for the subsequent studies, the wheat bran inoculum would be more suitable at these levels, and that the disease scoring system would be appropriate.

CHAPTER 5

INFLUENCE OF ZN NUTRITION ON SEVERITY OF *RHIZOCTONIA* ROOT ROT OF WHEAT GROWN IN A CONTROLLED ENVIRONMENT

5.1. INTRODUCTION

The next and most important step in studying the effect of an environmental factor on disease severity is to set up experiments which involve various levels of the factor under controlled conditions (Colhoun, 1973; Graham, 1983). In Chapter 2, it was demonstrated under field conditions that severity of *Rhizoctonia* root rot was significantly lower in plants with higher Zn concentration. In the preliminary pot experiment discussed in Chapter 3, in spite of some technical limitations, the result indicated that increasing Zn concentration in the plant tissue by the application of Zn to Zn deficient soil could reduce percentage of seminal roots infected by the fungus; that is, Zn deficiency might be the cause of increased disease severity. More suitable inoculum levels and rates of Zn for the Zn-*Rhizoctonia* system were established as described in Chapter 4.

All of the earlier studies described in Chapters 3 & 4 led up to the experiment reported in this chapter in which Zn and inoculum treatments at levels previously optimized in Chapter 4 were combined factorially in a detailed study under controlled conditions. The experiment is designed to test the following hypotheses: (i) that the severity of the disease depends on the Zn concentration in plant tissue, so increasing the Zn concentration in plant tissue, through applying Zn to Zn deficient soil, reduces the severity of *Rhizoctonia* root rot; (ii) that Zn deficiency is the cause of increasing disease severity by predisposing the wheat plant to *Rhizoctonia* root rot and aggravating disease severity. Furthermore, the experiment aimed to quantify the extent of the interaction on plant growth between inoculum level and applied Zn, so the levels of both factors which

provide the strongest suppression of lesions could be identified.

This experiment is expected to support, not only the evidence from field and the previous pot experiment of a correlation between Zn nutrition and this disease, but also to test the hypothesis of a causal link behind the observed correlation. Furthermore the discovery of the levels of both Zn and inoculum at which the effect on disease is strongest should generate some speculation on the nature of the resistance mechanism.

5.2. EXPERIMENT 5: MATERIALS & METHODS

5.2.1. *Experimental design.* A factorial experiment was set up with six levels of added Zn (0, 0.01, 0.04, 0.1, 0.4 and 6 mg Zn/kg soil represented by Zn_0, $Zn_{0.01}$, $Zn_{0.04}$, $Zn_{0.10}$, $Zn_{0.40}$ and $Zn_{6.0}$) and 4 inoculum densities (0, 2, 4 and 8 propagules/kg soil represented by I_0, I_2, I_4 and I_8) with 4 replicates. These treatment levels were chosen as a result of the previous experiments in Chapter 4.

5.2.2. *Inoculum.* Wheat bran was used as a food base for the fungus because it gave a better range of disease severities than millet seed. The inoculum was prepared according to the method described in Chapter 4.

5.2.3. *Soil & pots.* Laffer sand, a Zn-deficient siliceous soil as used in all pot experiment in Chapters 3 & 4 was used after leaching using the procedure previously described. Dry soil weighing 500 g (560g wet soil at field capacity) was used for each polypropylene pot, 15 cm high and 7 cm diameter.

5.2.4. *Basal nutrient.* The basal nutrients were adjusted according to plant analysis of tissue in Experiment 4.2 in Chapter 4 to give a better balance of nutrients. The basal nutrients (mg/pot) were: 176 NH_4NO_3, 45.7 $MgSO_4.7H_2O$, 3.32 $MnSO_4.4H_2O$, 0.18 H_2MoO_4, 0.07 $CoSO_4.7H_2O$ and $NiSO_4.7H_2O$, 1.13 H_3BO_3 and 0.33 $FeSO_4.7H_2O$; the others remained as for the experiment in Chapter 3. A nutrient solution was prepared, using Milli-Q® water, which contained all of the nutrients except Zn. Zn solutions were prepared from a stock solution of 1M $ZnSO_4.7H_2O$

(analytical grade) to provide either 0, 0.005, 0.02, 0.05, 0.2 and 3 mg Zn in 1ml (the amount applied to each pot). $CaCO_3$ 0.3% w/w was mixed thoroughly with the dry soil before the nutrient solutions were added to the soil.

5.2.5. *Seed.* Seeds of Songlen wheat similar to those in Chapters 3 & 4 was used. They were surface sterilized and pregerminated as described before.

5.2.6. *Procedure.* The pot preparation was identical to the procedure for the experiment in Chapter 3. Dry soil (450 g) was thoroughly mixed with basal nutrient solution and 1 ml of a Zn solution according to treatment. The inoculum treatments were created by placing 0, 1, 2 or 4 pieces of wheat bran/pot in the soil, split between two layers of the soil as described in Chapter 3, except that the 1 propagule was put in the middle of the pot. All pots were then watered to 85% field capacity, which is 10.2% moisture content (w/w), and incubated in a controlled environment chamber where the light was supplied by banks of high-intensity metal-halide and incandescent lights which gave a light flux density of photosynthetically active radiation of 450 μmol. m^{-2} s^{-1}, set on a 10/14 h, 15 /10°C day/night cycle for 2 weeks before planting, to allow the fungus to colonize the soil.

After 2 weeks, three germinated seeds (as described in 5.2.5) were placed on the soil surface of each pot followed by a further 50 g dry soil; water was added to make 12% moisture content (w/w). The soil surface was covered with acid-washed black plastic beads to a depth of 1 cm to reduce water loss and algal growth.

The experiment was watered to field capacity regularly and left in the growth chamber under the conditions described above. At 21 days after planting, all the plants were harvested, and measurements taken for plant height and top fresh and dry weight. Root systems were assessed visually, using the scale developed by Dr G C MacNish (Sweetingham and MacNish, 1992), by reference to Figure 4.1 in Chapter 4. The scale from 0.5 to 5.0 in 0.5 increments is inversely related to the length of seminal root axes. Scores 0 and 0.5 have the same root length but 0.5 has clear evidence of lesions. The

plant tops were analysed for Zn concentration by ICP spectrometer, as previously described. All statistical analyses were performed using the GENSTAT 5 program (GENSTAT 5 Committee,1989).

5.3. RESULTS

Figure 5.1 shows growth in terms of height, top dry weight and root fresh weight. Statistical analysis of all of the growth parameters indicated that Zn and inoculum treatments did not interact but affected yield independently (Appendix 7). Growth appeared to be slightly decreased as inoculum increased, but the effect of inoculum, from the ANOVA, was not significant in the time frame of this experiment. The growth response to applied Zn showed 2 phases. Dry weights from treatments Zn_0, $Zn_{0.01}$ and $Zn_{0.04}$ were obviously lower than from the $Zn_{0.1}$, $Zn_{0.4}$ and Zn_6 treatments. There was a large response in top dry weight to increasing the Zn applied from 0.04 to 0.1mg. Other growth indices also showed a trend similar to that of top dry weight.

The response in growth to applied Zn corresponds to the decrease in disease severity (Figure 5.2). At all inoculum densities, the severity of disease was markedly decreased by applications of Zn at 0.1 mg/kg or more; the disease score at each inoculum density dropped sharply between treatments $Zn_{0.04}$ and $Zn_{0.1}$ (Figure 5.2), a difference which reflected the response patterns in growth (Figure 5.1). The disease score was halved by $Zn_{0.1}$ across all inoculum densities. Against this major trend there were minor trends in the reverse direction at Zn_0I_2: disease severity of $Zn_{0.01}$ treatments was generally higher than the Zn_0, and at I_8, the scores of the $Zn_{0.4}$ and Zn_6 treatment were also higher than in the $Zn_{0.1}$. Analysis of variance in disease score shows a highly significant interaction between Zn and inoculum treatments when the I_0 data are included (Appendix 7). While this interaction is meaningful and important biologically, the ANOVA is necessarily flawed by the lack of variance in the I_0 disease scores. Figure 5.3 shows that disease severity was significantly correlated to top dry weight.

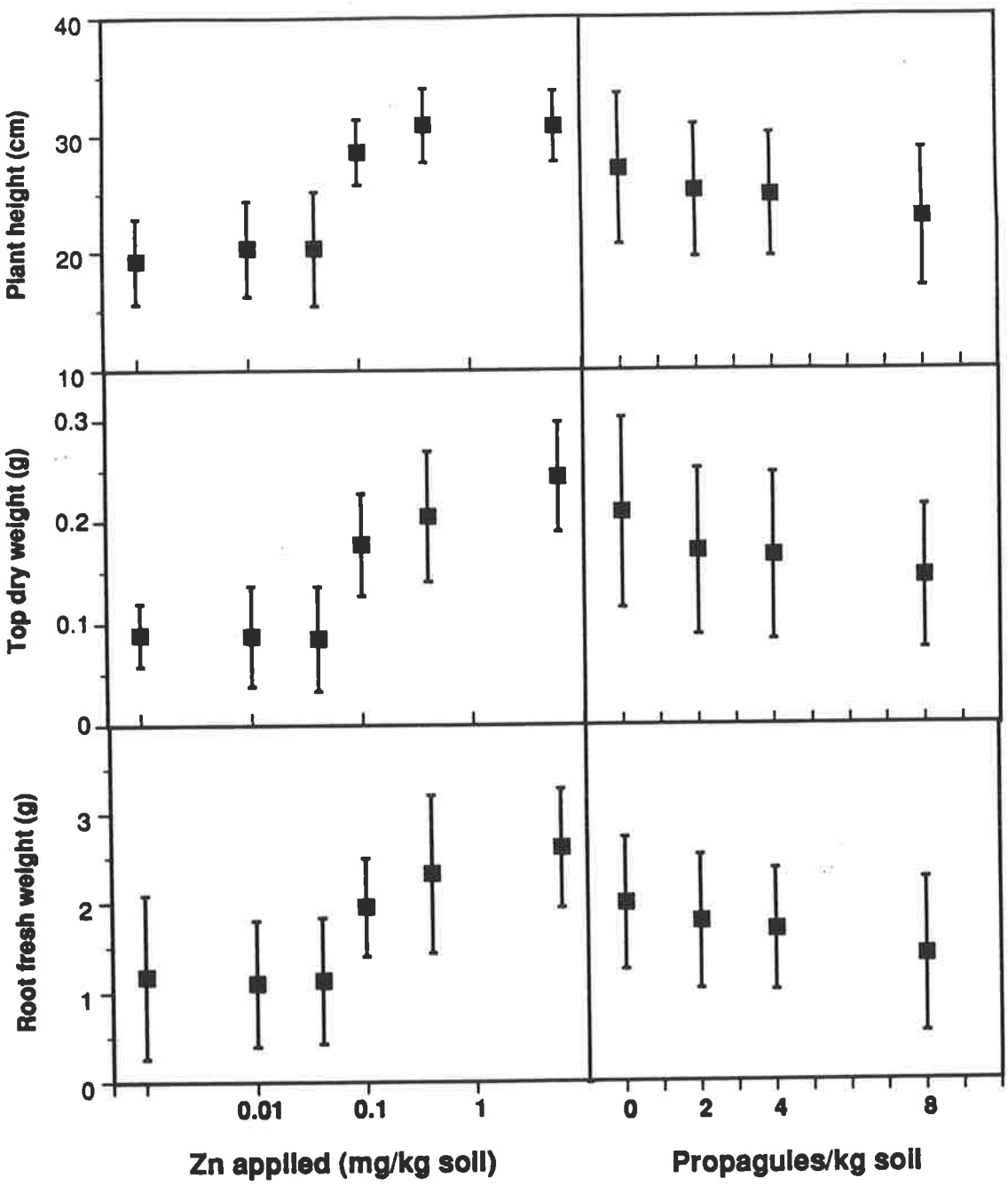


Figure 5.1 Growth of wheat plants, 23 days old in terms of height, top dry weight and root fresh weight, averaged over Zn (left column) and inoculum (right column) treatments. Note that the effect of Zn treatments is more obvious than the effect of inoculum treatments.

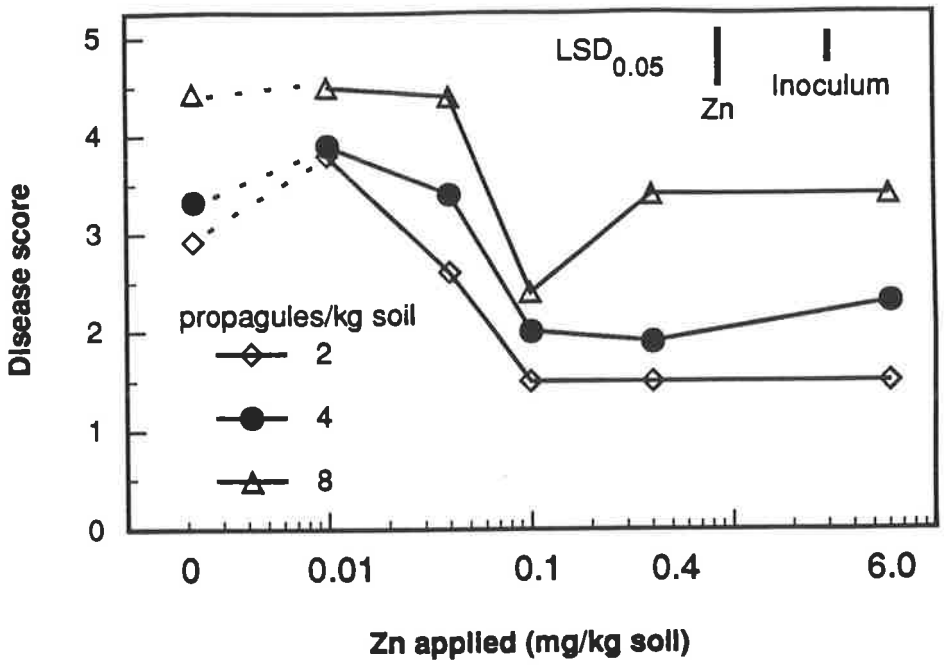


Figure 5.2 Disease severity at each inoculum density as a function of the level of Zn applied in log scale. Scoring system is as illustrated in Figure 4.4 of Chapter 4. LSD Zn is to compare a difference between two Zn means at the same inoculum level, and LSD inoculum to compare a difference between two inoculum means at the same Zn level .

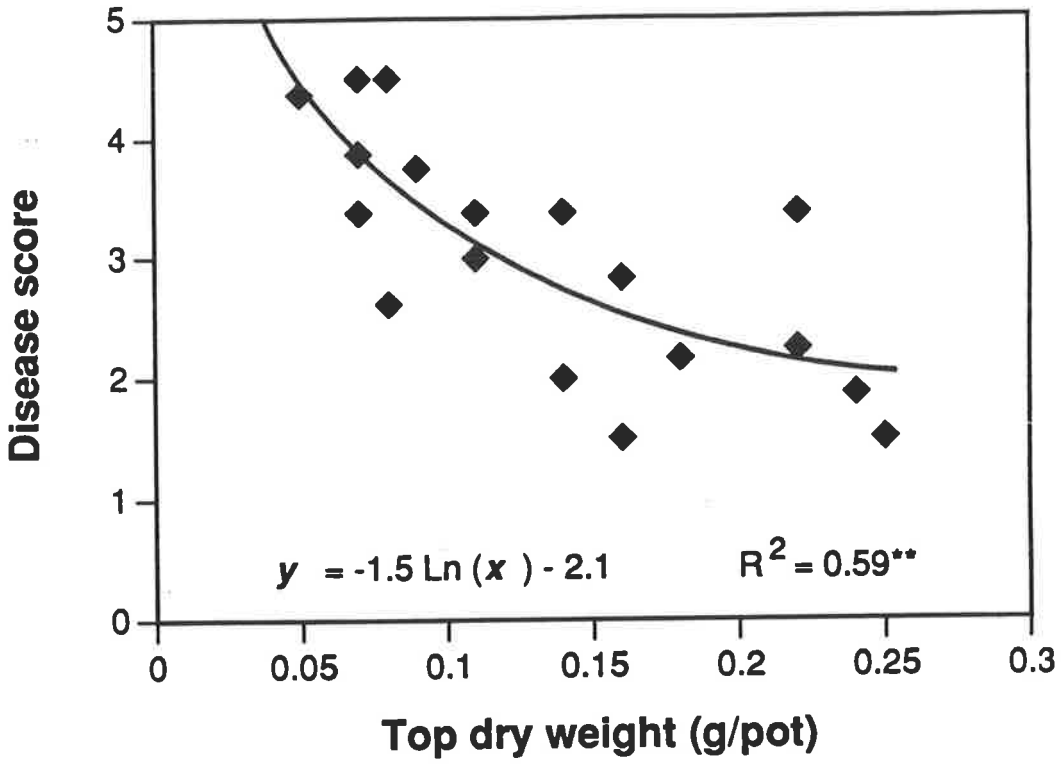


Figure 5.3 Significant correlation between disease severity and top growth of 23 day old wheat plants from the inoculated treatments in Experiment 5. Each point represents the mean over four replications.

The low dry weights at Zn0, Zn0.01 & Zn0.04 were associated with high disease scores (Figure 5.4A). With more than 0.04 mg Zn/kg soil, there was a sharp drop in disease score and an increase in plant dry weight. In addition to this, Zn concentration in the tops started to increase from around 10 mg/kg or less in Zn0, Zn0.01 & Zn0.04 treatments to around 20 mg/kg in Zn0.1 treatments (Figure 5.4B). Zn concentrations in shoots, illustrated in Figure 5.4B were significantly affected by Zn treatments but not by inoculum treatments, and a similar trend was observed with the Zn contents in plant tops since inoculum rates did not significantly affect top dry weight.

An attempt to correlate disease severity with Zn concentration in the plant, in the same manner as in the field, is illustrated in Figure 5.5. Even though the correlation was not significant because of the few data points involved, this experiment shows the same trend of reduction in disease severity with increasing Zn concentration in the plant.

Although there were insufficient Zn treatments to define the Zn critical concentration closely, maximum growth in the uninoculated treatment was again achieved between 15 and 25 mg Zn/kg (Figure 5.6). This is within the range of published data (Reuter and Robinson, 1986) which means that the technique used in our system is appropriate; that is, the levels of Zn used in this experiment spanned acute deficiency to adequacy, a suitable range for a system investigating a Zn-disease interaction.

5.4. DISCUSSION

5.4.1. FURTHER CONCLUSIONS ON THE SUPPRESSION OF DISEASE BY ZN

The levels of Zn in this experiment more effectively spanned the range from deficiency to sufficiency, according to the growth response curve, than those used in the experiments in Chapters 3 & 4. The $Zn_{0.1}$ treatment raised the Zn concentration in the plants to about the critical level and strongly decreased disease compared to the next lowest Zn level. This result is consistent with the application of 2.5 kg Zn/ha in the field

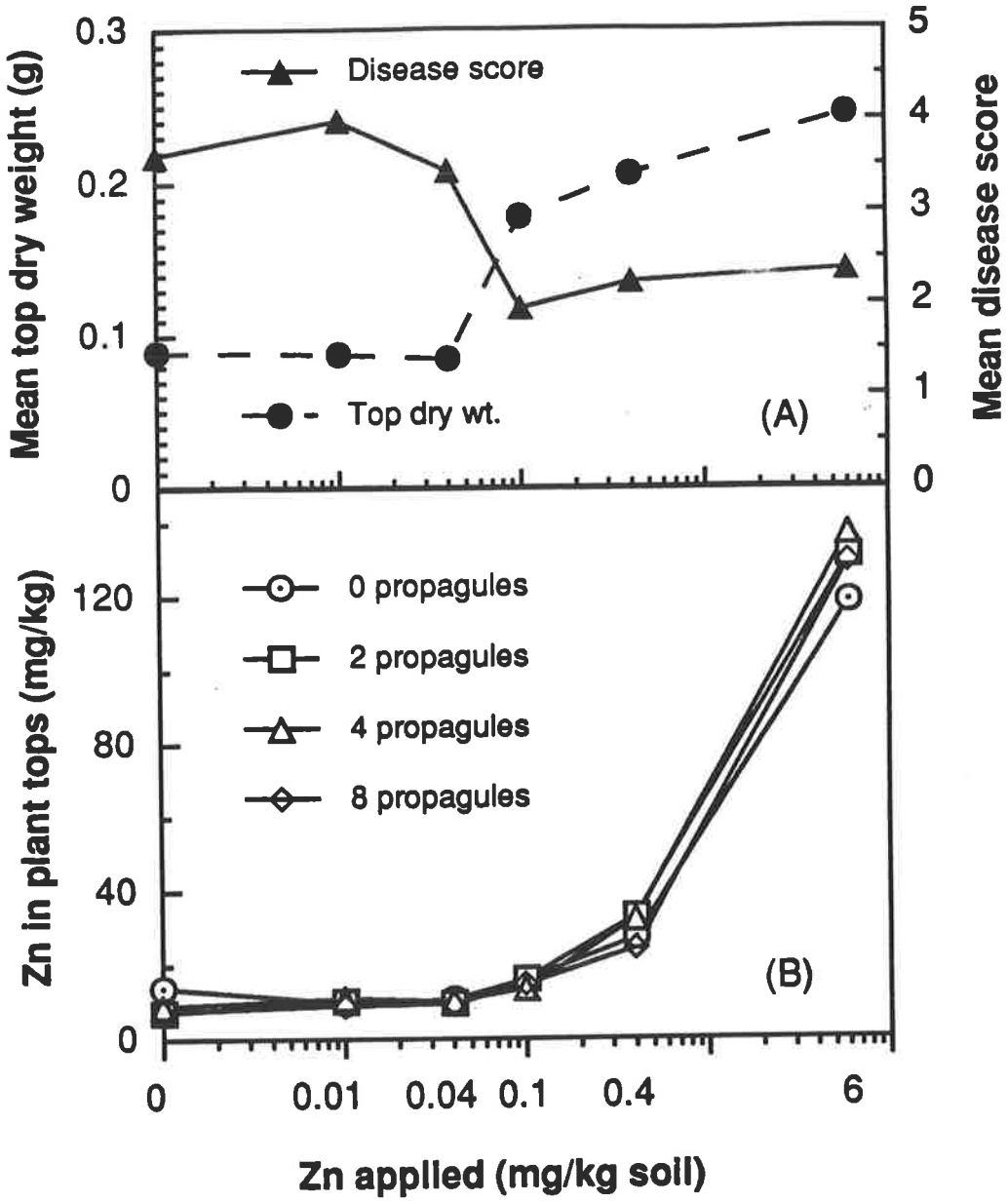


Figure 5.4 (A) Mean top dry weight (g) and mean disease score of Songlen wheat grown at six Zn levels (averaged over all inoculum levels) and (B) Zn concentration in plant tops according to rates of Zn applied to soil for each of four inoculum treatments.

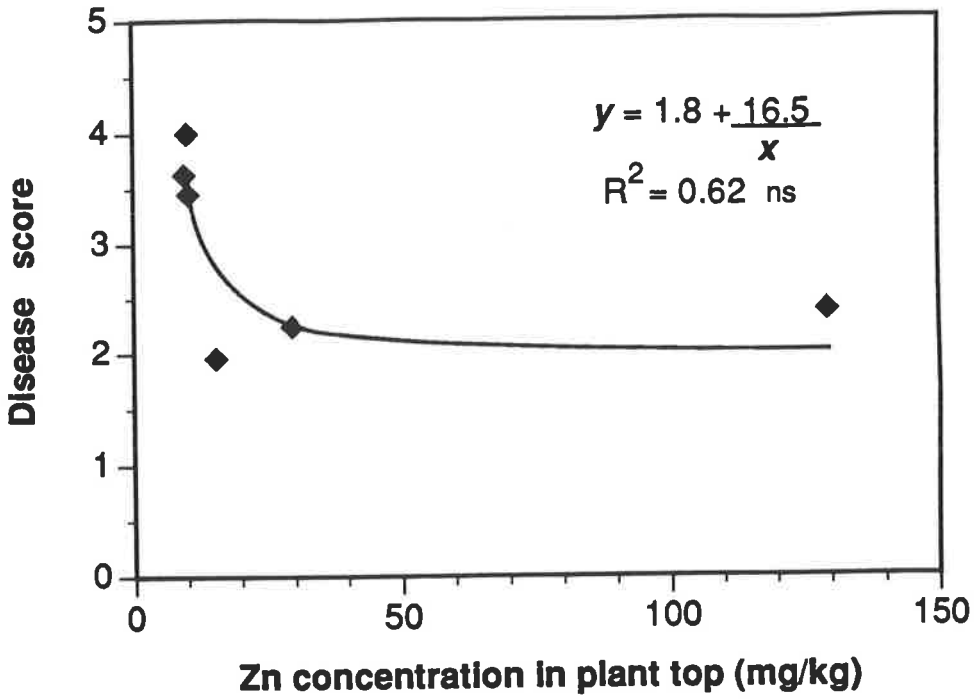


Figure 5.5 The curvilinear correlation between average disease severity and average Zn concentration in plant tops from the Experiment 5, in the same trend as in the field experiments in Chapter 2. Significant R^2 at P = 0.05 is 0.65.

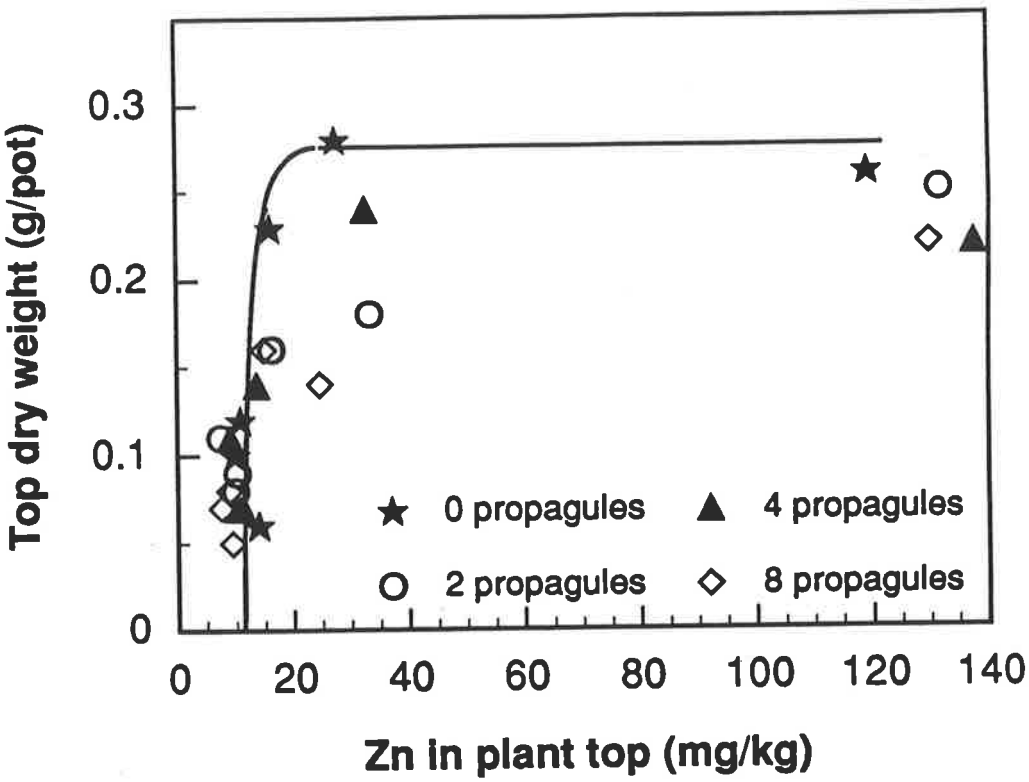


Figure 5.6 The relationship between top dry weight of wheat plants and the concentration of Zn in those tissues. The response curve was drawn for the uninoculated treatment only. The critical Zn concentration is in the range of 15-25 mg/kg.

(Experiment 2.2 of Chapter 2), and with the general hypothesis of Graham (1983) that nutritional effects on disease resistance occur over the deficiency range only. The $Zn_{0.1}$ level of Zn is in the sub-optimal to optimal range, so unlikely to be toxic to the fungus, and the effect of Zn is therefore most likely to be through physiological processes in the plant, as suggested by Graham & Webb (1991).

An important result from the experiment was the high severity of disease in plants that were deficient in Zn due to the low levels of available Zn in the soil. At the three lowest Zn application rates, the plants were Zn deficient; an addition of 0.1 mg Zn/kg to plants at this stage markedly reduced disease severity. Once the Zn status in the plant had been raised to an adequate level, further application of Zn showed little or no effect in decreasing disease severity (Figures 5.2 and 5.4A). This phenomenon was clearly demonstrated both in the field and in controlled conditions. In Experiment 2.2 of Chapter 2, disease severity in terms of %BPA was not significantly reduced further by Zn applied at rates higher than 2.5 kg/ha which is the rate that just corrected Zn deficiency in the plants. Results from the experiment in this chapter also confirm this point.

Most previous studies have reported effects of Zn on disease to be due to the direct toxicity of high soil Zn levels on the pathogen living as a saprophyte (Wilkinson & Millar, 1981; Somashekar *et al.*, 1983; Cripps *et al.*, 1983; Hooley & Shaw, 1985; Haque & Mukhopadhyaya, 1983; Savor, 1986; Somani, 1986). There is not much work with low or modest levels of Zn that range from deficiency to sufficiency. Bolle-Jones & Hilton (1956) showed more *Oidium heveae* infection in Zn-deficient rubber leaves. Tomlinson (1958) found that 0.5 mg/kg of $ZnSO_4$ could inhibit growth of *Spongospora subterranea* that caused crook-root disease of watercress by killing zoospores of the fungus and, later, Tomlinson & Hunt (1987) showed that sub-optimal Zn supply at the rate of 2 mg Zn/L could suppress both crook root and chlorotic leaf spot virus (WCLV) in watercress through *Spongospora subterranea*, which is the vector of WCLV. Finally, Sparrow & Graham (1988) demonstrated that a low Zn application of 0.06 mg/kg could limit the upward progression of *Fusarium graminearum* through the stele of the wheat

plant.

The zinc-induced decrease in disease severity demonstrated in these experiments extends the earlier observation of Millikan (1938), the results from the field from Chapter 2, and also the results of the preliminary pot experiment in Chapter 3. The effect of Zn in reducing disease severity is more obvious at lower levels of inoculum than at medium or high levels. This may imply that Zn cannot induce total resistance to the disease, a condition typical of disease-nutrient interactions described by Graham and Webb (1991). This trend is similar to those involving the interaction of Mn and the take-all fungus, caused by *Gaeumannomyces graminis* var *tritici,* reported by Wilhelm (1991). These results imply that, although there are demonstrated effects of nutrition on disease suppression, the effect may vary with the level of inoculum in the soil. Graham & Webb (1991), for example, state that the nutritional effects of disease suppression are greatest when the potential of the inoculum to infect a plant, or the inoculum density, is modest.

Another interesting point from this experiment is that disease severity is not at its highest in the Zn_0 treatments. It may be possible that the fungus also suffered from Zn deficiency in the Zn_0 treatment and was therefore not as active as in the next two higher levels of Zn. This speculation needs to be verified by further investigation.

5.4.2. Confirmation of a Causal Link Between Zn Concentration and Disease Severity

There were no statistically significant differences in either Zn concentration or Zn content of shoots among the three inoculum levels within the same Zn application, but there were great differences in disease severity between Zn treatments at the same inoculum level. This result established that increasing disease severity through the inoculum treatments did not lower Zn concentration in the plant tissue, but increasing Zn concentration in the plant did reduce disease severity. Once again, these results support the statement in Chapter 3 that Zn deficiency aggravates severity of *Rhizoctonia* root rot in cereals, rather the alternative pathway of induction of greater Zn deficiency by

increasingly severe disease. Finally, it could be concluded that, in the Zn-*Rhizoctonia* relationship, Zn deficiency is the predisposing factor, initiating disease by predisposing the plant to the disease.

This is the first time that cause and effect in the Zn-*Rhizoctonia* interaction has been established systematically. The first evidence supporting this concept of nutrient deficiency-induced disease severity may have been reported by Vanterpool (1940, 1952), who proved from his study that phosphorus deficiency was the primary cause of browning root rot of wheat by *Pythium* spp. in Canada; the disappearance of this disease is due to adequate use of phosphatic fertilizer. Graham and Rovira (1984) presented similar evidence for the role of manganese in resistance to take-all disease caused by *Ggt*, and Sparrow and Graham (1988) also established this argument with Zn and susceptibility to crown rot caused by *Fusarium graminearum* Schw. Group 1. In all cases, the interaction occurred in nutrient deficient soil in which the plants responded to the application of the limiting nutrient.

The evidence supports the statement of Garrett (1970) about 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."

5.4.3. Comparison of Results from Field and Pot Experiments in the Correlation Between Zn Concentration in Plant Tops and Disease Severity

Correlation between the reciprocal of Zn concentration in plants and disease severity in this experiment, from Figure 5.6, was significant at $p<0.1$ ($R^2=0.62$), close to the figure of $R^2=0.65$ at which significance would have been $p<0.05$. Significance can hardly be expected in a regression of six points. However, the greater significance of the correlation from the field compared to the pot experiments may be not only because of

much greater number of points, but may also be because of the different nature of the root environment in field and pots. This could be considered in a number of ways. Firstly, there is a larger plant root population in the field which may provide more opportunity for random infection than in the pot. Secondly, there are higher variations in growth and vigour of both plants and fungus in the field; there is the possibility that some strong plants will be attacked by inoculum from a weak propagule base and infection will not develop, or *vice versa*. Thirdly, application of 2.5 mg Zn/ha in the field providing the maximum plant growth response is estimated to be equivalent to about 1 mg Zn/kg soil (for the soil, with estimated bulk density of 1.3 g/cm^3 to 15 cm depth) whereas in the pot experiment, that rate fell at only 0.1 mg Zn/kg soil, ten times lower than in the field. It might be that a better distribution of Zn through the soil in the pots provided better contact between Zn in the soil and the absorption sites at the root surface, so that most of the Zn applied could be more readily available for uptake by the plants compared to application of Zn in the field. This higher and more even supply of Zn may mean fewer opportunities for escape or else limitations to infection as described under the second consideration mentioned in this paragraph.

The more random opportunities for infection and the variations of both plants and fungus in the field could also explain the difference in disease severity and infection between experiments in pots and in the field; accordingly, percent seminal roots infected (%SRI) may be an effective index to illustrate degree of disease severity in the field, but less so in pots. This index makes it difficult to conclude whether the Zn effect in decreasing disease severity operates by increasing plant "tolerance" or plant "resistance" to the disease. According to Agrios (1988), resistance is the ability of a host plant to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor, while tolerance is the ability of a host plant to sustain the effect of a disease without dying or suffering serious injury or crop loss. A good example of the effect of nutrients on host tolerance to disease was reported for the relationship between Mn deficiency and cereal cyst nematode (CCN) in barley by Wilhelm *et al.*(1983). They demonstrated that adding Mn to barley plants grown in Mn deficient soil did not reduce

the invasion of CCN into the plant roots but the growth of the plants with added Mn was not reduced by this level of infection. On the other hand, reports of Graham & Rovira (1984) on Mn and take-all interaction, and Sparrow & Graham (1988) on Zn and *Fusarium* crown rot interaction clearly showed less spread of the pathogen in the plants supplied with those nutrients, and established that the effect of nutrients was on the host resistance.

Based on the fact that *R. solani* damages the plant by degrading the root cell wall, which causes the symptoms of cortical rotting and root-pruning, it might be concluded that the plant is not affected by the fungus if there are no symptoms appearing in the root system. Therefore, resistance to the disease might be determined by none or fewer symptoms of disease on the root systems. Results from all studies in the field Experiments 2.1 and 2.2 in Chapter 2, pot Experiment 3 in Chapter 3 and Experiment 5 of this chapter demonstrated that the plants with higher Zn concentration had a lower percentage of infected roots and/or a lower disease score. These results suggest that the higher concentration of Zn in the plant tissue increases plant "resistance" to the disease. This type of resistance may be classified as a "horizontal resistance" in which the plants are not totally protected from becoming infected but the degree of severity is decreased, and with which there need be little association with the genotype of the plant (Vanderplank, 1968; Zadocks & Schein, 1979; Agrios, 1988).

Speculation on the mechanisms involved in the resistance is the subject of the remaining parts of this thesis.

CHAPTER 6

SPECULATION THE MECHANISMS OF SUPPRESSION OF THE SEVERITY OF *RHIZOCTONIA* ROOT ROT BY ZN

The results of all the experiments, either in the field or in pots (reported in Chapters 2, 3, & 5), indicated that plants with higher Zn concentrations were less diseased than plants with lower Zn concentrations, and the application of Zn at rates that just lifted Zn status in the plant from deficiency to adequacy could decrease the severity of *Rhizoctonia* root rot on wheat plants growing in Zn deficient soil. These results run parallel to the evidence from Graham & Rovira (1984), supported by works of Rovira *et al.*(1985) and Wilhelm (1991), on suppression of the severity of take-all disease by applying Mn to Mn-deficient soil at a rate sufficient to correct Mn-deficiency in plants, and of Sparrow and Graham (1988), who demonstrated that Zn application at 0.06 mg/kg into Zn-deficient soil could curtail the upward progression of *Fusarium graminearum* through the stele of wheat plants.

According to Baker (1965) and Baker *et al.* (1967), disease severity has two components, 'inoculum potential' and 'disease potential'. Inoculum potential is the pathogen's capacity to colonize and infect at the surface of the substrate, and is a result of inoculum density and environmental factors affecting the inoculum. Disease potential is the ability of the host to contract a disease, which is a function of its susceptibility at different growth stages and the effects of environment. Zn, as one of the environmental factors affecting severity of *Rhizoctonia* root rot, may have effects either on the plant's physiology (that is, on the disease potential component) or on the fungus (the inoculum potential component of disease severity). The diagram in Figure 6.1 shows the link between all of these components and some speculated mechanisms which are mentioned below.

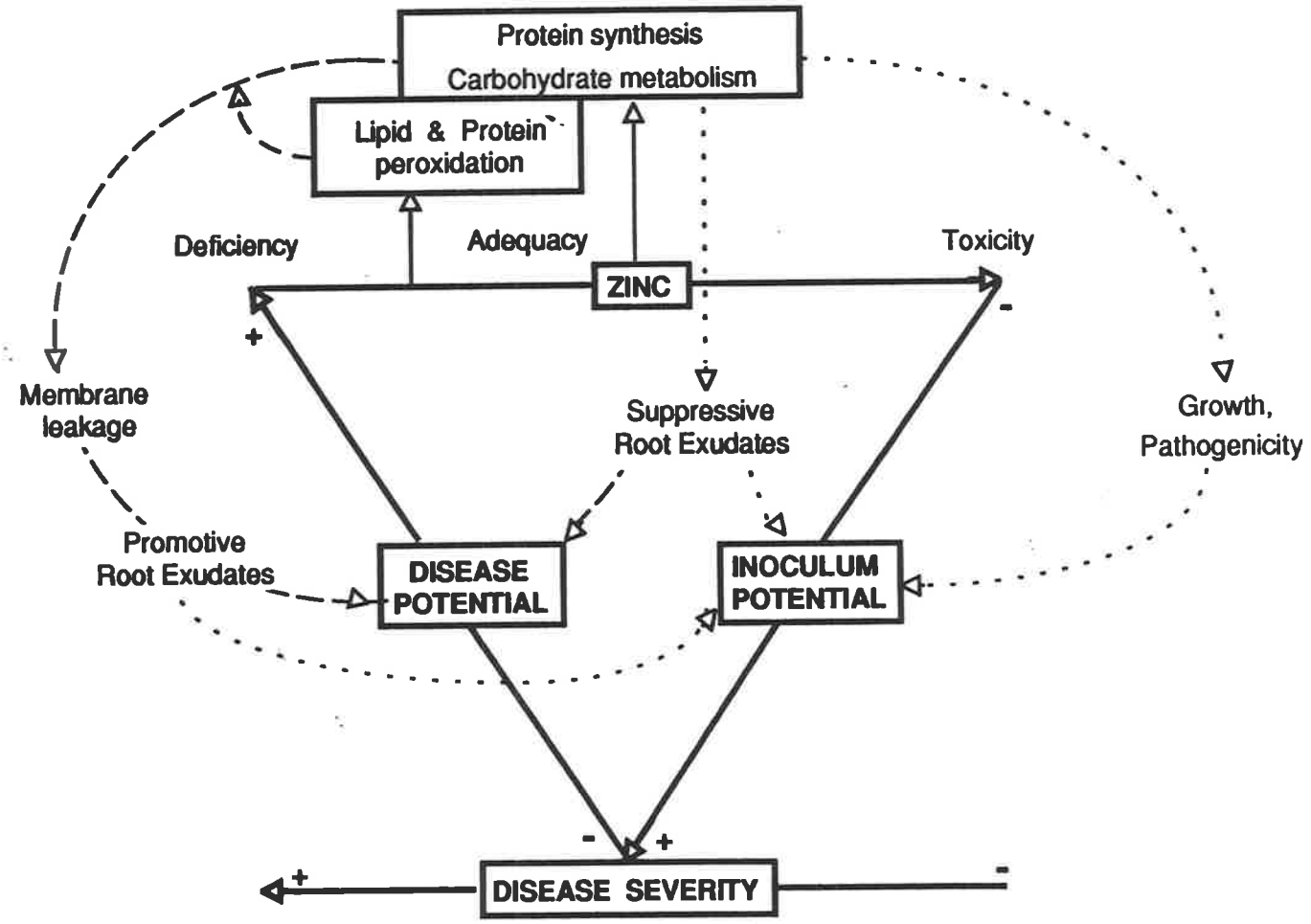


Figure 6.1 Diagram illustrating Zn-*Rhizoctonia* root rot relationships. Explained are the link between Zn, disease severity, disease potential and inoculum potential, together with speculated mechanisms.

Possible mechanisms of suppression of the severity of *Rhizoctonia* root rot by Zn are as follows:

6.1. ADVERSE EFFECT OF ZN SUPPLY ON GROWTH AND/OR PATHOGENICITY OF THE PATHOGEN

Although the levels of Zn supply in this study are considered to be in the suboptimal to optimal range, and too low to be so toxic that they inhibit growth of the fungus in the soil, it is possible they may decrease growth and/or the virulence of the fungus in attacking the plant roots. Moreover, the relatively low level of infection in the nil Zn treatment in the pot experiment of Chapter 5 suggests that the fungus itself might suffer from Zn deficiency, thereby reducing the severity of infection compared to the lowest level of Zn addition. This hypothesis should be tested.

6.2. EFFECT OF ZN DEFICIENCY ON THE SUSCEPTIBILITY OF THE PLANTS

The alternative or complementary hypothesis is that the effects of Zn supply in the suboptimal to optimal range on suppression of *Rhizoctonia* root rot severity are due to physiological processes in the host plant. Since Zn is known to be involved in both protein and carbohydrate metabolism and, perhaps more importantly, in the integrity and stability of the biomembrane, possible mechanisms for higher susceptibility in Zn deficient plants may be suggested as follows:

6.2.1. THE ROLE OF ZN IN THE INTEGRITY OR STABILITY OF THE HOST PLANT'S MEMBRANE

Zn-deficient plants contain abnormally low levels and activity of the enzyme superoxide dismutase and, therefore, high levels of superoxide radical (O_2^-) (Cakmak and Marschner,1988), which lead to peroxidation of membrane lipids, loss of membrane integrity and increased membrane permeability (Thompson *et al.*,1987). In addition, the

accumulation of free amino acids and amides occurs as a result of inhibition of protein synthesis by Zn deficiency (Steinberg 1956 cited by Shkol´nik, 1984). These two effects together may result in an increase in free amino acids in the root exudate of Zn-deficient plants owing to the leakiness of the membrane. Thus, the infection by the fungus may be accelerated by the chemotaxic effects on the fungus of the organic and inorganic substances in the root exudates of Zn-deficient plants, as Flentje *et al.* (1963) showed that root exudation is important for the infection process of *R. solani.*

6.2.2. THE ROLE OF ZN IN CARBOHYDRATE METABOLISM

There is a number of reports on impaired carbohydrate metabolism in Zn deficient plants, evidenced by the accumulation of reducing sugars in parallel with a reduction in sucrose, polysaccharides and starch (Shkol´nik, 1984, Marschner, 1989). Pectin, one of the cell wall components, was reported to have high metal binding capacities (Camire & Clydesdale, 1981). Veeranjaneeyulu & Das (1982) demonstrated in both leaves & roots, by using ^{65}Zn isotope, that there was high concentration of Zn accumulated in the cell wall pectic components. Karakis *et al.* (1989) found that Zn was strongly bound to the carboxylic groups of pectic substances in pea roots. As carbohydrate is a basic component of cell walls, any limitation may have direct effects in weakening the wall of root epidermal cells, especially pectic components in the cell wall, and may make them more susceptible to fungal infection. For *R. solani*, infection is reported to be due to the activity of cell wall degrading enzymes, especially pectic enzymes (Flentje *et al*, 1963, Beateman & Miller, 1966; Beateman, 1967; Lai *et al,* 1968; Kommedahl & Windels, 1979).

The first speculated mechanism, that of a direct effect of Zn on the fungus, is hypothesized and tested in Chapter 7; the remaining mechanisms are discussed in the Chapter 8.

CHAPTER 7

EFFECT OF ZN ON THE SAPROPHYTIC GROWTH OF *R. SOLANI* KÜHN

7.1. INTRODUCTION

Results from the field and pot experiments in Chapters 2, 3, and 5 showed an obvious reduction in the severity of *Rhizoctonia* root rot with increasing Zn application. According to Chapter 6, one of the hypotheses for the suppression of *Rhizoctonia* root rot severity by Zn application is a direct toxic effect of Zn on the fungus. Therefore, a series of experiments, described in this chapter, was conducted to examine the effect of Zn on growth of the fungus in the absence of a host plant.

It was also the purpose to explain, if possible, why, in the pot experiment of Chapter 5, the severity of disease increased a little at low rates of Zn application before decreasing markedly at Zn rates above $Zn_{0.04}$. The hypothesis is that the low rates of Zn stimulated the saprophytic growth of the fungus in the soil making it more virulent. This proposition embodies the assumption that the fungus has a lower external critical requirement for Zn than does the host, so that it reaches maximum vigour at a lower level of Zn than the host. To this end, the culture media, using ordinary agar at first and thereafter a purified agar, were used to assess the effects of Zn on the fungus. Thus, the first studies were in artificial culture medium. Finally, growth of the fungus in soil was measured across a range of levels of applied Zn similar to that used in the pot experiment of Chapter 5 so that the low levels of infection in the nil Zn treatment of the pot experiment in Chapter 5 possibly could be explained.

7.2. EFFECT OF ZN ON THE GROWTH OF *R. SOLANI* IN SYNTHETIC MEDIA

7.2.1. MATERIALS AND METHODS

In the following two experiments, the effect of Zn on the growth of *R. solani* was tested. The fungus was grown in two agar media, differing in the levels of Zn impurity, with 10 different levels of added Zn : 0, 0.001, 0.01, 0.02, 0.05, 0.15, 0.6, 1.5, 6.0 and 15 mgZn/15 mls agar. These corresponded to 0, 0.01, 0.1, 0.2, 0.4, 1.2, 4.8, 12.0, 48.0 and 120 mgZn / kg soil (on the basis of the water present), covering the range used in the pot experiment of Chapter 5 in order to simulate the Zn status of that experiment. Both experiments were arranged in a Completely Randomized Design with 3 replications. Detailed procedures of these two experiments are as follows.

7.2.1.1. Experiment 7.1

7.2.1.1.1. *Agar medium.* The synthetic agar used in this experiment was a weak Czapek-Dox medium plus yeast extract (1/6 NDY: Appendix 3). The agar was prepared using ordinary agar (Sigma's Agar-Agar powder which contained 4 µg Zn/g agar - by ICP analyzer) and Milli-Q® water; adjusted pH to 7; and then 14 mls were put into each of 30 small vials, which was the total number of plates used in this experiment.

7.2.1.1.2. *Zn solution.* Zn solutions of 10 concentrations, according to the treatments mentioned in Section 7.2.1, in the separate vials (10 mls per vial) were prepared from a stock solution of 1M $ZnSO_4.7H_2O$ and Milli-Q® water.

7.2.1.1.3. *Procedure.* Thirty vials of prepared agar medium (Section 7.2.1.1.1) and 10 vials of Zn solutions (described in Section 7.2.1.1.2), were autoclaved at 120°C and 100 kPa for 15 minutes. They were then left to cool to about 45°C in a laminar flow cabinet before being poured into plastic petri dishes (9 cm diameter). To pour a plate, a vial of 14 ml agar was poured into a petri dish, then 1 ml of Zn solution

was added using a sterile micropipette. The plate was gently shaken so that the two solutions were thoroughly mixed and then left in the laminar flow cabinet until the agar had cooled and set.

Pure culture of *R. solani* isolate no. R21 provided by Dr. S.M. Neate was used as in the previous pot experiments. The fungus was cultured in the sterile 1/6 NDY agar, as previously described, in sterilised petri dishes at 15°C for 4 days before starting the experiments. Mycelial discs, 0.5 cm diameter, taken from edge of the actively growing colonies, were used as inocula for the test dishes. One mycelial disc was aseptically transferred to the centre of each dish, and then the dishes were kept in the dark at 15°C for 7 days. The colony diameters were measured at regular intervals using the average of measurements along four random diameter lines each time.

7.2.1.2. Experiment 7.2

The materials and procedure were the same as in Experiment 7.1 except that purified agar (Sigma^{TM}'s Agar-purified, plant cell culture tested grade with 1 µg Zn/g measured by ICP analyzer) was used in the 1/6 NDY agar instead of the ordinary agar used in Experiment 7.1.

7.2.2. RESULTS

The colony diameters obtained from Experiments 7.1 and 7.2 are shown in Figures 7.1A & 7.1B, respectively. In Experiment 7.1, increasing the rate of Zn applied had no effect on either daily or final colony diameter until the Zn concentration was raised to 0.01 mg Zn/plate, when the final growth of fungus was half of that in the other treatments. Growth was completely inhibited by the application of 0.6 mg Zn/plate.

The same trend is shown in Experiment 7.2 (Figure 7.1B); however, fungus growth started to be reduced at a Zn application of 0.05 mg/plate. Again, it was Zn at 0.6 mg/plate that completely stopped growth of the fungus.

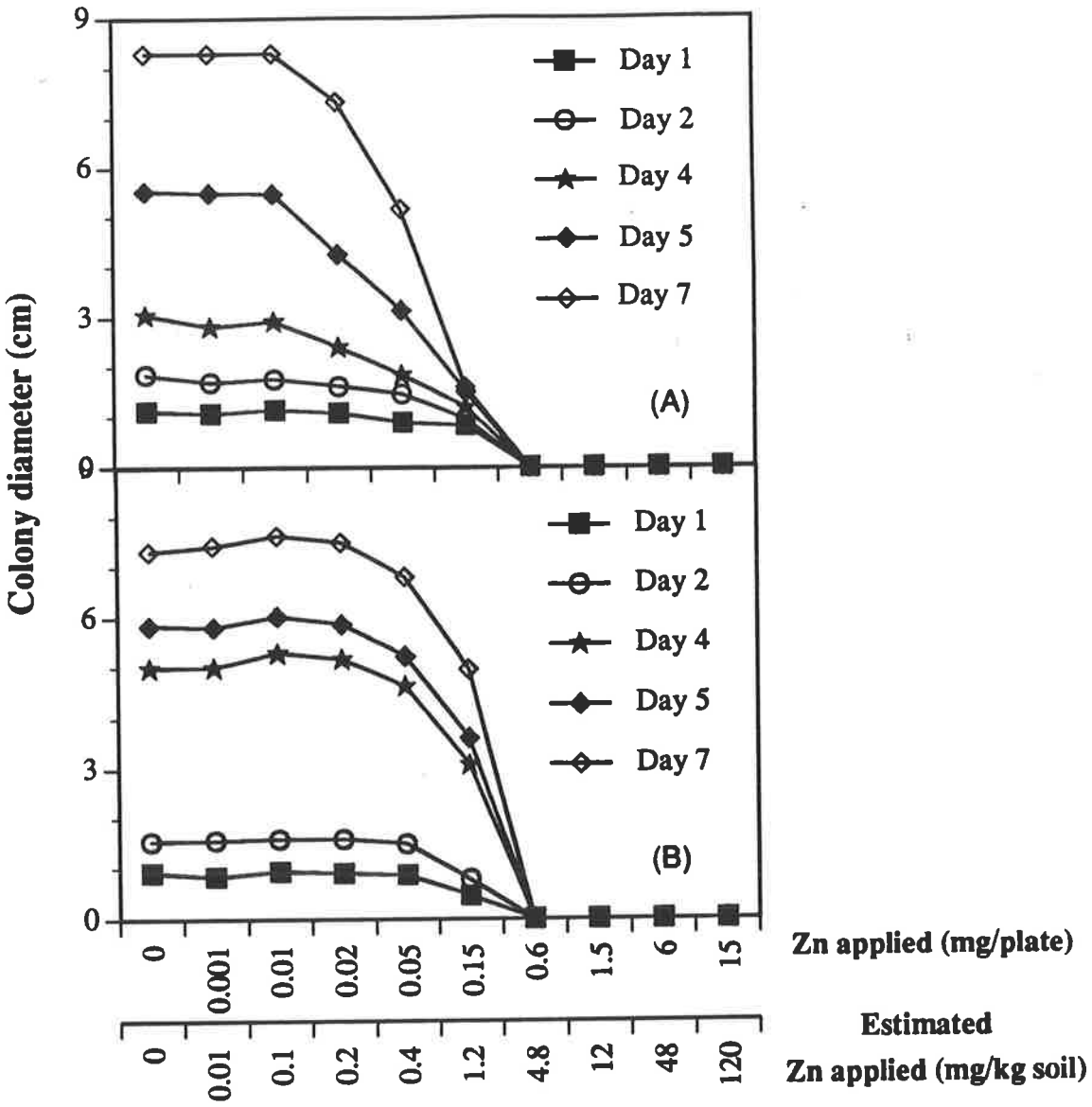


Figure 7.1 Growth of *R. solani* (AG8) at 10 different rates of Zn in 1/6 NDY medium using (A) ordinary agar in Experiment 7.1, and (B) purified agar in Experiment 7.2. The measurement of colony diameter was averaged over three replicates on days 1, 2, 4, 5 and 7.

These results show that Zn applied to the agar media completely inhibited growth of *R. solani* at the rate of 0.6 mg/plate. This rate of Zn was estimated to be equivalent to 4.8 mg Zn/kg soil (see Figure 7.1), at which, in pot Experiment 5, the fungus was still active in infecting the roots, since root rot symptoms could still be detected. It may be that all Zn applied to the agar was available whereas, in the soil, some was fixed and unavailable. This inconsistency of results of these experiments relative to the pot experiment of Chapter 5 led to studies of the fungus growth in a soil system, similar to that used in the pots.

7.3. EFFECT OF ZN LEVELS ON GROWTH AND DEVELOPMENT OF *R. SOLANI* IN SOIL

Results from the agar media system in Section 7.2 showed that growth of *R. solani* was stopped completely at a Zn application of 0.6 mg Zn/plate. This rate of Zn is estimated to be equivalent to 4.8 mg of Zn applied to 1 kg of soil whereas, according to the results from Experiment 5, the wheat roots were still infected even at a level of 6.0 mg Zn/kg soil. This suggested that studying the effect of applied Zn on fungal growth in agar might not be appropriate to explain the behaviour of the fungus in the previous pot experiments using soil; therefore, fungal growth was observed in the same soil as that used in the pot system, in another three experiments as follows.

7.3.1. MATERIALS AND METHODS

The 10 levels of Zn: 0, 0.01, 0.1, 0.2, 0.4, 1.2, 4.8, 12.0, 48.0 and 120 mg Zn/kg soil were used to test the effects of Zn on growth of *R. solani.* Laffer sand was washed with RO water as described in Chapters 3 &5. These rates are equivalent to Zn: 0, 0.001, 0.01, 0.02, 0.05, 0.15, 0.6, 1.5, 6.0 and 15 mg Zn/15 mls agar from the agar media experiments. To visualize growth of the fungus, three different methods were used in different experiments as described below.

7.3.1.1. Experiment 7.3

Procedure. Dry soil (30 g/plate) was thoroughly mixed with 0.3% $CaCO_3$ and put in a 30ml plastic vial. The basal nutrients and Zn solution (according to the treatments in Section 7.3.1), were then applied to and mixed with the soil. The basal nutrients (mg/30 g soil) were: 10.6 NH_4NO_3, 3.7 K_2SO_4, 4.0 KH_2PO_4, 2.75 $MgSO_4.7H_2O$, 0.2 $MnSO_4.4H_2O$, 0.02 H_2MoO_4, 0.3 $CuSO_4.5H_2O$, 0.004 $CoSO_4.7H_2O$ and $NiSO_4.7H_2O$, 0.042 H_3BO_3 and 0.001 $FeSO_4.7H_2O$, which were equivalent to the amounts in 500 g soil used in Chapter 5. The moist soil was then packed at a bulk density of 1.3 kg/dm^3 into a 90 mm plastic petri dish and a single wheat bran propagule was inoculated onto the middle of the plate. The plates were incubated in the dark for 2 weeks under the same incubation conditions used in the pot experiments.

Assessment for growth of the fungus was attempted by direct isolation of the fungus from the soil on 2, 4, 5 and 7 days after incubation. In the laminar flow cabinet, 4 soil pellets were taken from each plate aseptically, using a sterilised dentist's amalgam gun, and put on a plate of Ethanol-Potassium nitrate medium (Trujillo *et al.*, 1987) (Appendix 3). These plates were then incubated under the conditions mentioned previously.

The method failed to generate hyphal growth from any of the soil pellets. Thus, the following two experiments were set up.

7.3.1.2. Experiment 7.4

In this experiment, growth of *R. solani* was established from the development of the colony on a Durapore® filter, using a modified soil sandwich technique (Grose *et al.*,1984), as follows:

a) After the moist soil was packed in the Petri dish, a Durapore ® filter was placed on the soil surface. Sufficient sterile Milli-Q® water was added on top of the

filter to establish good contact between the filter and the soil surface, and to result in a soil water content of 85% of field capacity, that is 10% w/w soil water content. A wheat bran propagule was then placed on the centre of the filter.

b) After incubating the plates for 5 days under the conditions mentioned previously in Chapter 5, the growth of the fungus was detected by staining the filters with Tryphan blue and allowing them to dry out in the laminar flow cabinet. The diameter of each colony was recorded using the average from eight measurements across each filter.

7.3.1.3. Experiment 7.5

The materials, methods and procedures were the same as in experiment 7.4 with the modifications that no filter was used and the propagule was just placed onto the soil surface in the middle of the plate. The fungal growth was observed every day using a compound microscope (x100). Measurements were made from the end of the mycelia visible on the soil surface and those points were marked on the top of the petri dish. The colony diameter for each day was measured from the mark, using the average from eight measurements for each plate. At the end of 7 days, when the fastest growing colony reached the edge of the plate, the colony diameters were measured from the mycelia from the top of the plate using a compound microscope, and from underneath the plate using the Wilovert® Inverted Microscope (x100).

7.3.2. RESULTS

Figure 7.2A & 7.2B show the diameter of *R. solani* colony measured at the end of Experiments 7.4 and 7.5, respectively. Statistical analysis showed that colony diameters from both experiments were not affected by increasing Zn except for possible toxicity at 120 mg Zn/kg in Experiment 7.5.

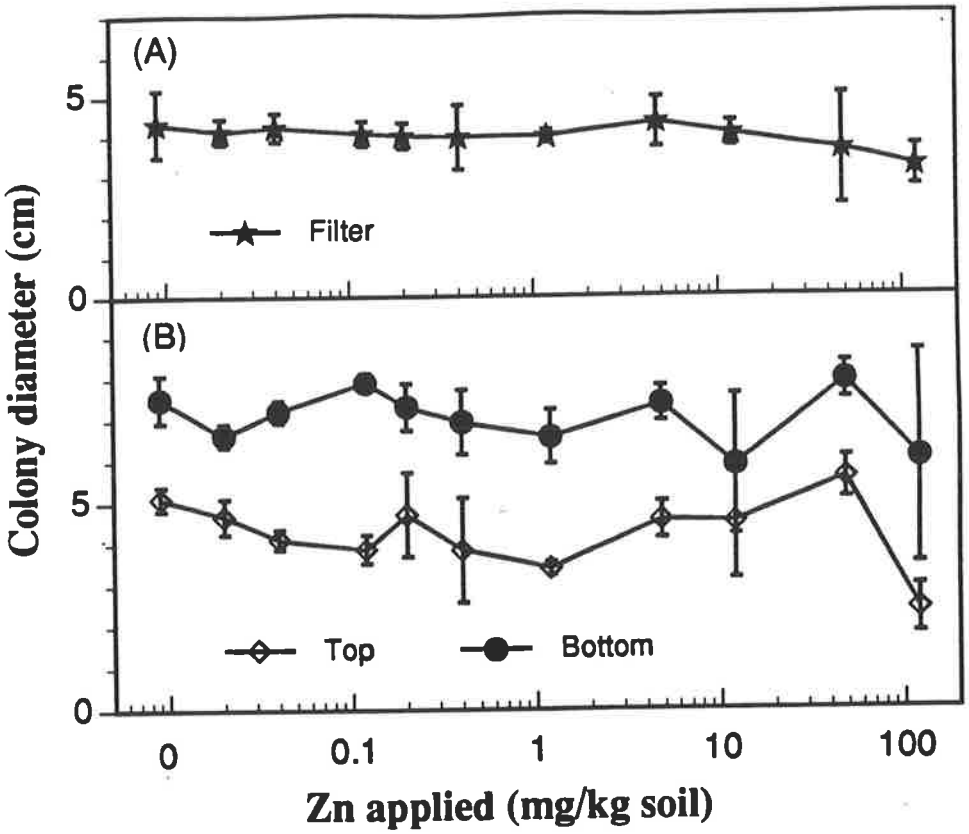


Figure 7.2 Growth of ***R. solani*** at 10 different Zn application rates 7 days after inoculation, measured as diameter of colony (A) on the Durapore filter in Experiment 7.4 and (B) by direct microscope observation of the top and bottom of the plate in Experiment 7.5. Error bars represent standard diviation within a Zn treatment.

7.4. DISCUSSION

Most research on the effect of Zn on *R. solani* suggests that a high Zn level may act as a fungicide and inhibit growth of the fungus; for example, McCarter & Barksdale (1977) reported that maneb-Zn applied to soil at the rate of 8.1 kg/ha reduced *Rhizoctonia* fruit rot in tomato; Babich & Stotzky (1978) found 10 mM Zn^{2+} completely stopped growth of *R. solani* in an agar medium, and Somani (1989) demonstrated that dipping potato tubers in 0.05% $ZnSO_4$ plus 1% acetic acid for 15 min, reduced the incidence of potato black-scurf caused by *R. solani*. However, results from the experiments in soil showed that fungal growth did not respond to increasing Zn levels to 120 mg Zn/kg soil.

This rate of Zn application is, however, reported to be in the toxic range for plant growth (Reuter & Robinson, 1986; Marschner, 1989). The toxic effect of Zn on plant growth was also confirmed in the experiments presented in Chapter 3 and in Experiment 4.1 in Chapter 4 when the application of only 12 mg Zn/kg soil showed toxic effects on wheat plants. Besides, the highest rate of Zn used in the pot experiment in Chapter 5 was only 6 mgZn/kg soil which, from the experiments in the soil systems in this chapter, had no effect on reducing the growth of the fungus. Therefore, according to our results, it could be suggested that an effect of Zn on suppression of *Rhizoctonia* root rot disease illustrated in the field and under controlled conditions must be due to the physiological effect of Zn on the cereal plant, rather than a fungicidal effect of Zn on the saprophytic phase of the fungus.

At the rate of 0.6 mg Zn/plate, equivalent to 4.8 mg Zn/kg soil, which is the Zn level that fungus growth was totally stopped in agar in Experiments 7.1 & 7.2, there were still some infected roots in Experiment 5. This indicates that, in the agar medium, the Zn supplied is present in relatively more available forms, compared to the soil system, in which the soil buffering capacity, high pH and $CaCO_3$ fix some Zn in unavailable forms (Lindsay, 1972). The actual concentration of available Zn in soil compared to the concentration in the agar medium is lower at the same rate of Zn application. This

speculation seems to be validated by the results from the soil system where the fungus seems to have slightly less but sustained growth at higher rates of Zn application than it does in the agar system.

The Zn impurity in the agar may also have to be taken into account. Steinburg (1950), when he reported from his experiment that the fungus achieved maximum growth at 0.4 mg Zn/kg, grew the fungus in still nutrient broth and purified only the reagents used in his medium. The amount of Zn that can be detected in most agars ranged from 12.3 µg Zn/plate in PDA, 0.6 µg Zn/plate in the ordinary agar to 0.3 µg Zn/plate in the purified agar. This amount of Zn, although lower than the lowest Zn addition, could have a significant impact on such a small colony of fungus growing in each plate. This view was confirmed by a slight difference in growth in the ordinary agar in Experiment 7.1 compared to that in the purified agar in Experiment 7.2. As illustrated in Figure 7.1, colony diameter on day 4, 5 and 7 in Experiment 7.1 started to drop at an application of 0.01 mgZn/plate, which is lower than in Experiment 7.2 where colony diameter started to drop at an application of 0.05 mgZn/plate. This difference suggests that, in addition to the Zn applied as treatments, there must be a considerable amount of Zn impurity in the ordinary agar that could be released and available to the fungus, resulting in a higher actual Zn concentration than in the medium with purified agar. That is why experiments in soil needed to be brought in for this type of testing in order to achieve a Zn status which could be directly related to those in the earlier pot experiments.

It seems that the technique of recovering fungal growth from soil by direct sampling (Experiment 7.1) is not valid for detecting the continuous growth of *R. solani* in the soil, even though it was reported by Van Bruggen & Arneson (1986) to be more effective than the wet-sieving technique. The poor results may be due to the severing of the mycelium from the food-base by cutting the soil with the amalgam gun in our experiment. This may provide a similar effect to tillage operations in the field. From our results, it might be concluded that the soil-pelleting technique is not appropriate as an non-destructive method of detecting the continuous growth of *R. solani* in a soil system.

Results were inconclusive about the suitability of the soil sandwich technique, although it was successfully used by Grose *et al.* (1984) for studying growth of *Gaeumannomyces graminis* var. *tritici* (*Ggt*) in soils. Unlike *Ggt,* mycelium of *R. solani* is colourless, does not leave any trace on the normal filter paper and, therefore, staining is needed to detect the colony diameter. Besides, Garrett (1962) reported that *R. solani* could decompose filter paper. The Durapore® filters seem to serve this purpose quite well but they are quite expensive. Furthermore, at the highest level of Zn, there was a growth depression detected by direct measurement which was not seen in the soil sandwich technique, perhaps because of poor contact between the fungus and the Zn in the soil via the Durapore® filters. Ultimately, the success of direct observations of the fungus by microscope made the complications and expense of the filter technique unnecessary for this fungus which has bright white shiny mycelium crossing soil pores.

However, this result still does not answer the question of why the severity of the disease increased a little on application of very low rates of Zn, before decreasing markedly with rates above $Zn_{0.01}$, in the pot experiment of Chapter 5 as illustrated in Figure 5.2. Results from all experiments in this chapter clearly demonstrated that growth of the fungus in either agar or soil with no Zn applied was not lower than at the higher Zn treatments. Therefore, although the fungus clearly has a lower external critical requirement for Zn than does the host, and in fact has maximum vigour at nil added Zn whereas the host requires 0.1 mg Zn/kg soil added, this does not explain the increase in disease at very low rates of Zn supply. It may be that, even though Zn deficiency in the growing medium had no effect on the growth of the fungus, it may reduce the pathogenicity of the fungus in some metabolic way and therefore reduce the infection. This speculation will be discussed in detail in the general discussion section.

It can be concluded from these results that Zn over the concentration ranges tested in the experiments either in this chapter or in Chapter 5 had little or no direct effect on growth of *R. solani* . Therefore, the suppression of disease severity at 0.1 mgZn/kg soil illustrated in the pot experiment in Chapter 5 is likely to be through physiological

processes in the plant leading to greater resistance, which will be discussed in the following chapter.

CHAPTER 8

GENERAL DISCUSSION

The data presented in this thesis provide the basis for more detailed studies leading to a better understanding of the role of Zn in plants and of the development of more effective control measures for *Rhizoctonia* bare patch. Some of the points will be discussed in this chapter.

8.1. IMPLICATIONS OF THE ZN-*RHIZOCTONIA* RELATIONSHIP FOR CROP HUSBANDRY

Attempts have been made to control *Rhizoctonia* bare patch disease for a long time. Fungicides were tested in agar and pots or as a seed-coating, but results are still inconclusive, especially for field work (Cotterill & Ballinger, 1989; Cotterill *et al.*, 1989; Smiley *et al.*, 1990a; Smiley *et al.*, 1990b). Screening for resistance found no consistent genetic differences among cereal cultivars (Purss & Dubé, 1984; McDonald & Rovira, 1985; Neate, 1989). Unlike take-all disease, the damage to wheat roots of *Rhizoctonia* has not been reduced in wheat grown after oats, medic pasture or peas in the rotations, and neither medic nor peas in the rotation could totally eradicate the area lost to patches caused by *Rhizoctonia* in wheat in the following season (Rovira, 1986; 1990). There was a report by Cowling & Sivasithamparam (1990) on biological control of *R. solani* by *Verticillium biguttatum* using infested soil from the field but the experiment was conducted in pots, and effective control was shown only in the treatments where *V. biguttatum* was mixed through the soil, so it was doubtful whether the effect of soil disturbance on the fungus might be involved and confounding the conclusions.

8.1.1. ZN-*RHIZOCTONIA* RELATIONSHIP AND INTEGRATED MANAGEMENT TO CONTROL THE DISEASE

At present, as previously mentioned, the only effective control for this disease is conventional cultivation before sowing (Bell, 1980; MacNish, 1983, 1985; Jarvis, 1984; Rovira & Venn, 1985; Jarvis & Brennan, 1986; Rovira, 1986; MacNish & Fang, 1987; Smith & Wehner, 1988; de Boer *et al.*, 1991). This practice can increase the risk of soil erosion in the wheat growing areas of southern Australia, particularly where the surface soil texture is sandy. Minimum tillage in the form of direct-drilling is recommended and increasingly being adopted, but it has been associated with an increased incidence of bare patch (Neate, 1984; Rovira, 1986; de Boer *et al.*, 1991). This complication has also occurred in some parts of USA such as the Pacific North West (Weller *et al.*, 1986), north eastern Oregon (Pumphrey *et al.*, 1987) and Georgia (Rothrock & Hargrove, 1987); and in South Africa (Smith & Wehner, 1988). The process of spraying herbicide to kill the grass pasture some weeks before sowing wheat in the direct-drilling system was suggested by Roget *et al.* (1987) to decrease *Rhizoctonia* inoculum but some herbicides such as chlorsulfuron (Glean®) were found to increase *Rhizoctonia* damage on wheat, and decrease yield (Rovira & McDonald, 1986). As *Rhizoctonia* root rot is widespread in the same areas where severe Zn deficiency and direct drilling occur concurrently, investigation of Zn management in that situation might lead to a more efficient direct drilling system adoptable by the farmers in those areas. Moreover, the report of Robson & Snowball (1990) that Zn deficiency was induced in wheat grown after the use of chlorsulfuron may, on the other hand, further strengthen the effect of Zn on the severity of *Rhizoctonia* bare patch in that situation.

In plotting the relationship between %BPA and Zn applied, the sharp change in the slope of the curve at the level of 2.5 kg Zn/ha in field Experiment 2.2 in Chapter 2 is useful information. This is the recommended rate of Zn application on wheat in the Mallee and Eyre Peninsula areas of South Australia where not only *Rhizoctonia* root rot,

but also Zn deficiency, are serious problems (Hannam, 1990). This result runs parallel to the result from the pot experiment in Chapter 5 where disease severity dropped sharply when Zn application was increased from 0.04 to 0.1 mg Zn/kg soil, which was also the point of maximum response of plant growth to Zn applied. These results imply that the same rate of Zn application may both correct Zn deficiency and minimize bare patch, and this should help farmers in these areas to adopt minimum tillage efficiently with a minimized effect from *Rhizoctonia*. Moreover, Zn application may be a worthwhile consideration in other areas of the world where *Rhizoctonia* bare patch is a problem.

8.1.2. Influence of Zn Contamination in the System of Screening for Disease Resistant Varieties

The discovery of an effect of Zn on disease severity might be useful in improving techniques for screening and breeding for disease resistant genotypes. Until now, breeders have not been successful in screening for genotypes of wheat or barley resistant to *Rhizoctonia* root rot. This disease is a pathogen dominant disease and the pathogen is nonspecialized, which means that the genetic resistance of the host is less important than the virulence of the fungus. Furthermore, the potential for Zn contamination in screening systems has been overlooked. It is possible that the reaction of the plant to the disease has been confounded by Zn present either in the seed or in the growing medium. Managing the level of Zn in the screening system might provide a better response in varieties tested for disease severity; that is, resistance may be expressed in Zn deficient soil and not in Zn sufficient soil.

Accordingly, whereas resistance to *Rhizoctonia* was not found in cereals under conditions which were probably Zn sufficient, it is hypothesized that differences in resistance linked to differences in Zn efficiency will show up when screened in Zn-deficient soil. This possibility already has a parallel in the Mn and take-all work of Wilhelm *et al.* (1990).

8.1.3. Zn-Efficient Cereal Varieties and Resistance to *Rhizoctonia* Root Rot

No disease resistant variety has yet been found. However, Zn-efficient genotypes (that is genotypes capable of extracting more Zn from deficient soil and maintaining considerable growth and yield in soil of low available Zn) are known to exist (Graham *et al.*, 1992; Graham & Rengel, 1993). These Zn-efficient genotypes might prove to be genotypes resistant to *Rhizoctonia* root rot in Zn-deficient soils because, compared with a Zn-inefficient genotype, they normally contain higher Zn concentration in their tissues which, based on results from this research, make the plants more resistant to the disease.

8.1.4. Zn Seed Content and the Severity of *Rhizoctonia* Root Rot in Seedling of Cereals

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 might be another means for protecting seedlings from *Rhizoctonia* root rot. Seeds with higher nutrient content, when grown in soil of low available nutrients, have been reported to produce more vigorous seedlings, better growth and higher yields than the low nutrient content seeds (Asher, 1987). There are also examples of B content in black and green gram seeds (Rerkasem *et al.*, 1990), Co content in lupin seeds (Robson & Snowball, 1987), Mn content of wheat seed (Singh & Bharti, 1985; Marcar & Graham, 1986) and barley (Longnecker *et al.*, 1991) and P content in wheat and medic seed (Bolland & Baker, 1988). These reports suggest the possibility of an effect of high Zn seed content in giving more vigorous seedlings and better Zn status, and consequently less disease infection.

8.1.5. ZN-*RHIZOCTONIA* RELATIONSHIPS IN OTHER PLANT SPECIES

Some of the major crops used in sustainable cropping systems such as grass pastures, medics, subterranean clover, peas and lupins were reported to be susceptible to *Rhizoctonia* root rot (Rovira, 1986, 1990). These crops also have been shown to be sensitive to Zn deficiency (Snowball & Robson, 1983; Hannam, 1990, 1991). Investigation of the Zn-*Rhizoctonia* interaction in those crops might provide information useful for sustainable cropping systems in the Zn deficiency-*Rhizoctonia* root rot infested areas.

8.1.6. METHODS USED TO ASSESS DISEASE SEVERITY IN THE FIELD

The current method of assessing disease severity is by examining damage to the root system to estimate the percentage of seminal roots infected, which is time and labour consuming in the processes of sampling, washing and assessing the root samples. These steps have to be taken as soon as possible after the root samples are collected, to avoid deterioration. Losing parts of the root system during sampling may also affect root scores for disease severity. No matter how carefully the root samples are collected, some loss is unavoidable, and this is greater late in the growing season when the root system is fully developed and the top soil has usually dried out.

Since 'bare patch' is the obvious above-ground symptom of *Rhizoctonia* root rot disease, there have been attempts to use it as a quick and more practical assessment of disease severity in the field, with fungus isolation from the roots to confirm the causal pathogen. This could be a better alternative to the current method previously mentioned. Percent bare patch area (%BPA) calculated from the hand-drawn map, even though time consuming, has been used and considered as an accurate index of *Rhizoctonia* bare patch area by many investigators (MacNish & Lewis, 1985; Jarvis & Brennan, 1986; MacNish & Fang, 1987; Cotterill, 1990). This method has been developed by Scofield (1919) & McNamara *et al.* (1931 cited by MacNish & Lewis, 1985) to investigate damage to cotton

from root rot disease caused by *Phymatotrichopsis omnivora* (Duggar) Henneb. Aerial photography has also been used (Bell ,1980 cited by MacNish & Lewis, 1985) but it is expensive, not always readily available, and still has some technical problems especially in identification of small patch areas or patches occupied by weeds. MacNish & Lewis (1985) reported a high correlation between percent bare patch area (%BPA), percent disease incidence (%DI) and percent appearance from patch scoring (comparable to our %PA), and suggested patch scoring to be the most convenient technique to investigate disease severity above ground. However, there was no correlation between %BPA and %SRI in their study. Their explanation was that the roots of the normal plants outside the patches were also infected and, thus, no correlation occurred between plant size and root damage. This also happened in our study. The seasonal variation previously described may also lessen the effect of both Zn deficiency and *Rhizoctonia* on plant growth. This may explain the failure to obtain significant correlations among methods used to measure *Rhizoctonia* bare patch in the field in 1990.

8.2. CONSIDERATION OF THE REMAINING MECHANISMS OF ZN-DEFICIENCY-INDUCED DISEASE SEVERITY

The experiments in Chapter 7 demonstrated that Zn had no direct effect on reducing fungal growth in the range of Zn from 0.04 to 0.1 mg Zn/kg soil. In that case, decreased disease severity from an application of 0.1 mg Zn/kg soil in the pot experiment in Chapter 5 was likely because of the change in the Zn status in the plant from deficiency to adequacy due to increase in Zn applied; this is clearly shown in Figure 5.3 of that chapter. This result pinpoints that it was not the toxic effect of Zn inhibiting the pathogen, but, on the other hand, it was the effect of Zn deficiency predisposing the plant to the disease and exaggerating disease severity. Therefore, the mechanism of Zn reducing severity of the disease is presumed to be due to the role of Zn in the physiology of the host plant.

According to Chapter 6, two physiological processes that might be involved in the susceptibility of the plant were hypothesized. One is the effect of Zn deficiency on root membrane leakage and the other is the effect of Zn deficiency on carbohydrate metabolism. Since the first mechanism has been well documented, it may be discussed in more detail.

8.2.1. EFFECT OF ZN DEFICIENCY ON ROOT MEMBRANE LEAKAGE & INFLUENCE OF ROOT EXUDATE ON FUNGAL GROWTH AND INFECTION

Effects of Zn deficiency on root membrane leakage and its influence on the amount and content of root exudates from Zn deficient plants have been previously discussed in detail in Section I.4.4 of Chapter 1. Here the influence of root exudates on the fungus will be considered.

It has been recognised for more than 100 years that root exudates have effects on microbial populations in the immediate vicinity of the root, or 'rhizosphere' (Rovira, 1956; Park, 1963; Rovira, 1965,1991; Foster *et al*, 1983; Curl & Truelove, 1986). Details of all these aspects were extensively reviewed by Hawes & Brigham (1992) as follows. Root exudates may control microbial populations both as promoters or as inhibitors. The promotive effects could be directly through the attraction of microbes to the rhizosphere, the induction of genes involved in plant-microbe associations, the suppression of microbial genes for dormancy and provision of nutrients for microbial growth, or indirectly, through factors such as mineralization of plant nutrients, stimulation of mutualistic microorganisms, structural support for growth of colonies, changes in the chemical environment like pH or inhibition of competing organisms. On the other hand, the inhibiting effects could be as direct as repulsion of the microorganisms from the rhizosphere, suppression of microbial genes required for the plant-microbe association, induction of microbial genes for dormancy, toxicity and/or antibiosis, or indirectly through the changes in chemical and biological environment of the rhizosphere,

effects on border cells and root structural changes that decrease mobility of the organisms.

The quantity and composition of root exudates vary according to the root region and between species of plants. Twenty-two amino compounds were found in the root exudates of 21 day old peas while only 14 were found in oats; besides, fructose, glucose and UV-absorbing and fluorescent compounds presented in the first 10 days (Rovira, 1956). Different substances were excreted from various regions of the roots. Significant amounts of threonine and asparagine have been found to be excreted from root tips, and leucine, glutamic acid and phenylalanine were excreted in great amounts from the root hair zone (Rovira, 1965). In Zn deficient plants, the plasma membrane permeability was increased as indicated by a 3-, 5-, and 2.5 fold increase in root cell leakage of K^+, NO_3^- and organic carbon compounds such as sugars, free amino acids and phenolics, respectively, compared to the Zn sufficient plants (Cakmak & Marschner, 1988a, b). The more nutritious root exudates from Zn deficient plants might attract and promote the fungus to grow and infect the root of such plants more than Zn sufficient ones as there is a number of reports on the stimulating effects of organic exudates on infection by *R. solani* (Kerr & Flentje, 1957; Flentje *et al,* 1963, 1964; Lewis, 1976; Dijst, 1987, 1988, 1990)

8.2.2. Effect of Zn Deficiency in *R. solani* on its Pathogenicity

According to the results from the pot experiment in Chapter 5, disease severity was not highest in the nil Zn treatment, an observation which was suspected to be due to the direct effect of Zn deficiency on the fungus. Although the nil Zn treatments in both agar media and soil were proven in Chapter 7 not to reduce fungus growth, it might still be the case that Zn deficiency decreased fungal pathogenicity through its effect on level of the pectolytic enzyme level or their activity in the fungus.

Production of extracellular pectolytic enzymes is one of the outstanding characteristics of *R. solani* which is used as criteria in its taxonomy to classify different

strains of this fungus (Sweetingham *et al.*, 1986; Neate *et al.*, 1988; Cruickshank, 1990). Matthew (1992) found that immunoreactive proteins were produced from AG-8 isolates of *R. solani* only in the presence of 0.1% pectin supplemented with 1% galactose or glycerol in the culture media, this being a specific characteristic of this anastomosis group of the fungus. It was previously mentioned that the infection mechanism of *R. solani* has been reported to depend on the cell wall degradation by these pectolytic enzymes such as polygalacturonases and pectin lyase. Ayers *et al.* (1966) suggested that susceptibility of snap bean to *R. solani* might be due to partial degradation of pectate by polygalacturonate *trans*- eliminase. Bugbee (1990) purified pectin lyase from *R. solani* group AG2-2 and showed that the wilt symptom of sugar beet plants could be reproduced by injecting the purified enzyme into the plant in absence of the fungus. This report runs parallel to the work of Flentje *et al.* (1963) which demonstrated development of necrotic symptoms in the roots of radish seedlings although the plants were separated from the fungus by using cellophane as a barrier. According to these reports, it might be implied that pectolytic enzymes produced by *R. solani* are responsible for the pathogenicity of this fungus.

Until now, there has been no report on the effect of Zn deficiency on pectolytic enzymes production and activity. However, as a result of the impairment of protein synthesis, Zn deficiency was reported to decrease both activity and level of numerous enzymes in other fungi (Shkol´nik, 1984). Therefore, Zn deficiency might have some effect on the pathogenicity of *R. solani* through the reduction of its pectolytic enzyme production and/or activity.

8.3. SUGGESTION FOR FUTURE RESEARCH

As this thesis is the pioneer report on the relationship between Zn deficiency and severity of *Rhizoctonia* root rot in cereals, the points previously discussed could be explored to elucidate this connexion and provide useful impact on both farming and scientific aspects.

8.3.1. APPLIED RESEARCH

1. Study of the resistance to *Rhizoctonia* of Zn-efficient cereal varieties in Zn deficient soils.

2. Study of the effect of Zn content in the seed on the severity of *Rhizoctonia* root rot in seedlings of the cereals.

3. Study of the relationship between Zn and *Rhizoctonia* in other plant species.

4. Study of the implications of Zn- *Rhizoctonia* interaction to the direct drilling system in the Zn-deficient areas.

5. Search for a fast and effective assessment of disease severity in the field

8.3.2. BASIC RESEARCH

1. Study the effect of Zn deficiency on root membrane leakage and influence of root exudate on fungus growth and infection.

2. Study effect of Zn on production and activities of pectolytic enzymes produced by *R. solani* and their effect on pathogenicity of the fungus.

CHAPTER 9

CONCLUSIONS

CONCLUSION ON ZN SUPPRESSION OF THE SEVERITY OF *RHIZOCTONIA* ROOT ROT IN WHEAT

This thesis is the first systematic attempt to investigate the relationship between Zn nutritional status and the severity of *Rhizoctonia* root rot of cereals in both natural and controlled conditions; the thesis has established a causal link behind the observed correlation and, finally, speculates on the possible mechanisms underlying this correlation.

From the results of all experiments described from Chapter 2 to Chapter 7, it may be concluded that:

a) In the field where Zn deficiency and *Rhizoctonia* root rot were concurrently widespread, Zn nutritional status of the cereal plant inversely correlated with the severity of *Rhizoctonia* root rot disease. The higher Zn concentration in the plant tops the lower the severity of disease in the roots.

b) Results from the pot experiments in Chapter 3 and 5 establish that *Rhizoctonia* root rot did not induce Zn deficiency in the plants but rather, Zn deficiency exaggerated the severity of the disease.

c). In both field trials in Chapter 2 and in the pot experiment in a controlled environment in Chapter 5, application of Zn to Zn deficient soil at rates up to those that raised Zn status in the plant from deficiency to adequacy, effectively minimized disease severity. Further application of Zn above an adequate amount was shown to have less effect in decreasing disease.

d) Two main mechanisms to explain the suppression of disease by Zn are proposed. Firstly, the effect of Zn might have been directly on the growth and/or pathogenicity of the fungus. Secondly and more likely, the effect of Zn might have been through physiological processes in the host plant. These processes were speculated to be an effect of Zn deficiency on the root membrane leakage or an effect of Zn on the pectic components of the root cell wall.

e). The first hypothesis of a direct effect of Zn on the fungus was tested in both agar media and soil, as described in Chapter 7. The results demonstrated that there was no effect of Zn in suppressing fungal growth in either agar or soil within the range of applied Zn from 0.04 to 6 mg Zn/kg soil that could explain the depression of disease severity in the plant according to the pot experiment in Chapter 5.

The diagram in Figure 8.1 was developed from Figure 6.1 to illustrate the relationship between Zn and severity of *Rhizoctonia* root rot and the postulated metabolic pathways involved, which were discussed earlier in this chapter.

In conclusion, these results establish that low Zn status in the plant, especially at levels of tissue concentration in the deficiency range, caused greater severity of *Rhizoctonia* root rot in cereals. Increasing Zn concentration level in the plant tissue up to adequacy decreased disease severity both in the field and in pots. Finally, it was not because of the direct toxic effect of increasing Zn application on saprophytic growth of the pathogen that the severity of *Rhizoctonia* root rot in wheat was reduced.

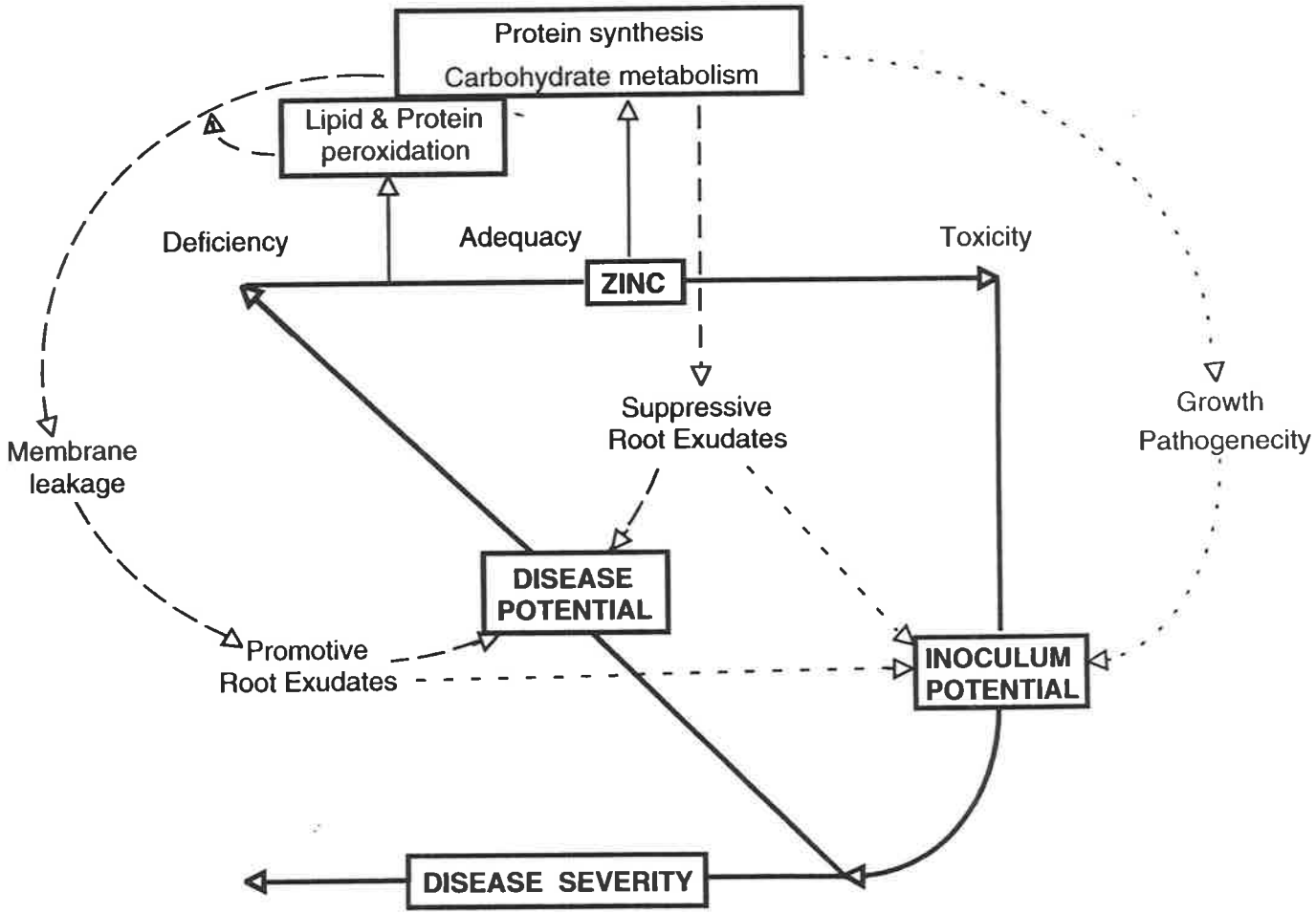


Figure 9.1 Diagram concluding the relationship between Zn and severity of *Rhizoctonia* root rot with the speculated metabolism involved. Details in Chapter 8, Section 4.

APPENDIX 1 Binomial test for the sampling data from Experiment 2.1.

Binomial test is the test of nominal data distribution unbiased among two catagories. It tests whether the proportion of individuals in one of the catagories, p´, from the observation falls within the confidence limits of binomial distribution. In this case, the two categories are to be or not to be infected by *R. solani.* Therefore, we used this test to determine whether the sampling plants have equal possibility to be infected by *R. solani,* which means that our random sampling procedure is unbiased. The null hypothesis is that each sampling has equal possibility to be infected by *R. solani.*

So, if p=probability of the samplings to be infected; then, Ho: p=0.5; Ha: p≠0.5

From the field survey; number of total samples, N = 60

Using the confidence limits as critical values (Zar, 1984):

According to Eqs. 22.26 and 22.27, we have

$$X = pN = (0.5)(60) = 30$$

Lower confidence limit, $$L_1 = \frac{X}{X+(N-X+1)\,F_{0.05(2),V_1,V_2}}$$

where $V_1 = 2[N-(X+1)] = 2[60-(30+1)] = 58$; $V_2 = 2X = 2(30) = 60$

From table B.4 (Zar,1984), $F_{0.05(2),58,60} = 1.676$

$$L_1 = \frac{30}{30+[60-(30+1)]1.676} = 0.381$$

Upper confidence limit, $$L_2 = \frac{(X+1)F_{0.05(2),V'1,V'2}}{X+[N-(X+1)]\,F_{0.05(2),V'1,V'2}}$$

where $V'_1 = V_2+2 = 60+2 = 62$; $V'_2 = V_1-2 = 58-2 = 56$

From table B.4 (Zar,1984), $F_{0.05(2),62,56} = 1.684$

$$L_2 = \frac{(30+1)1.684}{60-30+[60-(30+1)]1.684} = 0.635$$

From the sampling data, number of samplings infected by *R. solani*, x = 24

As the observed p´(=x/N=24/60=0.4) lies inside the range of 0.381 and 0.635, we accepted the Ho. So this set of data is distributed between inside and outside patches without bias.

APPENDIX 2 Summary of the multiple correlation for all nutrient elements and percent seminal root infected, from field experiment 2.1, 1989.

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Fe, Mn, B, Cu, Mo, Co, Ni, Zn, Na

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	9	8.23	0.9143
Residual	48	45.71	0.9523
Total	57	53.94	0.9463
Change	-9	-8.23	0.9143

*** Estimates of regression coefficients **

	estimate	s.e.	t
Constant	2.392	0.884	2.71
Fe	-0.000192	0.0008	-0.22
Mn	0.01399	0.00829	1.69
B	0.010	0.115	0.09
Cu	-0.101	0.144	-0.71
Mo	0.232	0.326	0.71
Co	-1.97	1.72	-1.14
Ni	-0.038	0.396	-0.10
Zn	-0.0399	0.0542	-0.74
Na	0.000033	0.000638	0.05

10 drop B

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Fe, Mn, Cu, Mo, Co, Ni, Zn, Na

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	8	8.22	1.0277
Residual	49	45.72	0.9330
Total	57	53.94	0.9463
Change	1	0.01	0.0071

Percentage variance accounted for 1.4

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	2.401	0.869	2.76
Fe	-0.000188	0.000851	-0.22
Mn	0.01387	0.00808	1.72
Cu	-0.095	0.118	-0.80
Mo	0.227	0.317	0.71
Co	-2.01	1.63	-1.24
Ni	-0.035	0.391	-0.09
Zn	-0.0383	0.0504	-0.76
Na	0.000053	0.000592	0.09

11 drop Ni,Na

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Fe, Mn, Cu, Mo, Co, Zn

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	6	8.21	1.3681
Residual	51	45.73	0.8966
Total	57	53.94	0.9463
Change	2	0.01	0.0064

Percentage variance accounted for 5.2

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	2.441	0.507	4.82
Fe	-0.000184	0.000793	-0.23
Mn	0.01339	0.00670	2.00
Cu	-0.090	0.110	-0.82
Mo	0.223	0.309	0.72
Co	-2.06	1.54	-1.34
Zn	-0.0388	0.0476	-0.82

12 drop Fe

1

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Mn, Cu, Mo, Co, Zn

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	5	8.16	1.6320
Residual	52	45.78	0.8803
Total	57	53.94	0.9463
Change	1	0.05	0.0483

Percentage variance accounted for 7.0

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	2.416	0.490	4.93
Mn	0.01359	0.00659	2.06
Cu	-0.100	0.101	-0.99
Mo	0.214	0.303	0.70
Co	-2.28	1.21	-1.89
Zn	-0.0372	0.0466	-0.80

3 drop Mo

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Mn, Cu, Co, Zn

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	4	7.72	1.9309
Residual	53	46.21	0.8720
Total	57	53.94	0.9463
Change	1	0.44	0.4367

Percentage variance accounted for 7.9

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	2.466	0.483	5.11
Mn	0.01188	0.00610	1.95
Cu	-0.0814	0.0972	-0.84
Co	-2.19	1.20	-1.83
Zn	-0.0269	0.0440	-0.61

14 drop Zn

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Mn, Cu, Co

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	3	7.40	2.4661
Residual	54	46.54	0.8618
Total	57	53.94	0.9463
Change	1	0.33	0.3252

Percentage variance accounted for 8.9

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	2.251	0.327	6.88
Mn	0.01052	0.00564	1.86
Cu	-0.0769	0.0964	-0.80
Co	-2.10	1.18	-1.78

15 drop Cu

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Mn, Co

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	2	6.85	3.4251
Residual	55	47.09	0.8561
Total	57	53.94	0.9463
Change	1	0.55	0.5482

Percentage variance accounted for 9.5

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	2.197	0.319	6.88
Mn	0.00772	0.00440	1.75
Co	-2.55	1.03	-2.48

16 drop Co

***** Regression Analysis *****

Response variate: SCORE: Fitted terms: Constant, Mn

*** Summary of analysis ***

	d.f.	s.s.	m.s.
Regression	1	1.60	1.6041
Residual	56	52.33	0.9345
Total	57	53.94	0.9463
Change	1	5.25	5.2460

Percentage variance accounted for 1.2

*** Estimates of regression coefficients ***

	estimate	s.e.	t
Constant	1.643	0.238	6.91
Mn	0.00595	0.00454	1.31

******** End of job. *********

APPENDIX 3 Media used for growing *Rhizoctonia solani*.

Czapek Dox (NDY) (Warcup, 1955)

	(g/l)	Comments
1. $NaNO_3$	2	
2. KH_2PO_4	1	
3. $MgSO_4$	0.5	
4. KCl	0.5	Autoclave 20 min at 121°C, 100 kPa.
5. $FeSO_4$	0.01	
6. Yeast extract	0.05	
7. Sucrose	30.0	
8. Davis agar	15	
9. Streptomycin sulphate		100 µg/ml
10. Tetracycline hydrochloride		50 µg/ml

1/6 NDY (Warcup, 1955) One-sixth of all ingredients above, except for agar which is full strength.

GN Medium (Trujillo *et al.*, 1987)

	/l	Comments
1. KNO_3	0.2 g	a) Mix 1, 2 and 3 and autoclave.
2. Agar	10 g	
3. Distilled H_2O	947 ml	
4. Ethanol 95%	21 ml	b) Mix 4 to mixture a).
5. Metalaxyl 250 EC	40 µl	c) Mix 5-8 with mixture b).
6. Prochloraz 40 EC	17.6 µl	
7. Streptomycin sulphate		100 µg/ml
8. Tetracycline hydrochloride		50 µg/ml

APPENDIX 4 Concentration of the other nutrient elements of wheat plants of Experiments 3, 4.1 and 4.2.

EXPERIMENT 3	NUTRIENT ELEMENTS (mg/kg)												
Treatments	**Fe**	**Mn**	**B**	**Cu**	**Mo**	**Co**	**Ni**	**Ca**	**Mg**	**Na**	**K**	**P**	**S**
Zn 0 i 0	118	139	31	17	18.3	0.43	0.79	6828	2503	148	5111	2024	3655
Zn 0 i 8	156	111	61	16	9.6	0.08	0.41	5124	2311	82	18505	2803	6471
Zn 0 i 16	179	134	62	30	5.9	0.11	0.59	4892	2317	117	28319	4249	9203
Zn 12 i 0	52	170	32	7.3	1.8	5.87	2.93	3597	1273	91	11986	1609	6756
Zn 12 i 8	45	160	30	7.0	2.2	3.36	1.78	3294	1133	70	10376	1316	5526
Zn 12 i 16	43	143	26	7.0	2.0	2.79	1.61	2994	1007	66	10634	1297	5183
	L	H							VL		L	VL	

EXPERIMENT 4.1	NUTRIENT ELEMENTS (mg/kg)												
Treatments	**Fe**	**Mn**	**B**	**Cu**	**Mo**	**Co**	**Ni**	**Ca**	**Mg**	**Na**	**K**	**P**	**S**
Zn 0 -Ca	104	148	26	14	21	0.18	1.14	5457	2053	52	6262	2782	3943
Zn 0 +Ca	102	183	20	13	23	0.13	0.92	5088	1430	41	5833	2385	3451
Zn 0.002+Ca	112	165	24	14	21	0.20	0.96	5082	1933	50	7275	2797	3734
Zn 0.004+Ca	91	193	22	13	23	0.10	0.84	5314	2028	47	5670	2242	3517
Zn 0.02 +Ca	79	123	15	11	18	0.15	0.79	3952	1671	47	7624	2510	3314
Zn12.0 +Ca	54	138	15	9	14	0.45	1.09	4085	1147	49	7104	2179	3145
		L			H				VL		VL	L	

EXPERIMENT 4.2	NUTRIENT ELEMENTS (mg/kg)												
Treatments	**Fe**	**Mn**	**B**	**Cu**	**Mo**	**Co**	**Ni**	**Ca**	**Mg**	**Na**	**K**	**P**	**S**
Zn 0	137	98	18	22	18	0.07	1.7	4695	2860	180	56302	24175	7875
Zn 0.01	150	97	23	25	14	0.50	1.8	4560	3022	187	63318	25632	6932
Zn 0.04	124	105	17	22	17	<0.05	1.2	4936	2898	143	59914	22188	6939
Zn 0.1	90	51	13	19	12	<0.05	0.6	3656	2057	185	56759	13600	6467
Zn 0.4	94	53	16	19	13	<0.05	1.0	3960	2172	200	54741	13744	6403
Zn 6.0	100	66	20	19	8	0.08	0.7	3847	2383	205	56762	16610	7417
				H	H							VH	H

APPENDIX 5 Growth, disease severity and Zn concentration of wheat plants 50 days old with two Zn levels and three levels of *R. solani* inoculum, Experiment 3.

Zn applied mg/kg	**Propa-gules** /kg soil	**Tillers /plant**	**YEBs with Zn def. symptoms**	**Height** cm	**Shoot dry wt.** g	**Root freshwt.** g	**Seminal root infected** %	**Zn in YEB** mg/kg
0	**0**	4.6	2.8	31	1.73	31.34	0	5.2
	8	2.7	2.0	34	0.76	6.98	98	7.0
	16	1.9	1.5	32	0.47	3.96	100	6.4
12	**0**	2.5	0	30	1.03	4.76	0	1110
	8	3.3	0.2	29	1.25	5.42	10	963
	16	2.6	0	30	1.23	4.98	25	834
ANOVA	Zn	ns	**	*	ns	**	**	**
	Inoculum	**	ns	ns	**	**	**	ns
	Interaction	**	ns	ns	**	**	**	ns

APPENDIX 6 Shoot dry weight (g) and percent of seminal roots infected, Experiment 3.
Analysed without the noninoculated treatments. Values in the brackets are the arcsine transformation.

Zn applied	Shoot dry weight (g)			% seminal roots infected		
	propagules/kg soil		Zn-mean	propagules/kg soil		Zn-mean
mg/kg soil	8	16		8	16	
0	0.76	0.47	**0.61**	98 (85.7)	100 (88.8)	**99 (87.3)**
12	1.25	1.23	**1.24**	10 (15.1)	25 (29.7)	**18 (22.4)**
Disease-mean	**1.01**	**0.85**		**54 (50.4)**	**63 (59.3)**	
LSD (P<0.05)		0.28			(9.3)	
(P<0.01)		0.39			(13.0)	

APPENDIX 7 Analysis of variance of disease score from Experiment 5 Analysed with and without noninoculated treatments.

Analysis of variance: with all inoculum treatments, log transformation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.31817	0.10606		
rep.plot stratum					
Zn	5	2.47658	0.49532	16.45	<.001
inoculum	3	33.32588	11.10863	368.97	<.001
Zn.inoculum	15	1.18216	0.07881	2.62	0.004
Residual	69	2.07740	0.03011		
Total	95	39.38019			

***** Tables of means *****

Grand mean 0.992

zn	0.00	0.01	0.04	0.10	0.40	6.00
	1.136	1.206	1.102	0.789	0.844	0.878

inoculum	0.00	2.00	4.00	8.00
	0.000	1.145	1.296	1.530

zn inoculum	0.00	2.00	4.00	8.00
0.00	0.000	1.382	1.464	1.698
0.01	0.000	1.543	1.577	1.703
0.04	0.000	1.277	1.455	1.674
0.10	0.000	0.906	1.085	1.163
0.40	0.000	0.862	1.046	1.468
6.00	0.000	0.896	1.146	1.472

*** Standard errors of differences of means ***

Table	Zn	inoculum	Zn x inoculum
rep.	16	24	4
s.e.d.	0.0613	0.0501	0.1227

Analysis of variance with polynomial contrast, all inoculation treatments, log transformation.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.31817	0.10606		
rep.plot stratum					
Zn	5	2.47658	0.49532	16.45	<.001
Lin	1	0.31640	0.31640	10.51	0.002
Quad	1	1.01864	1.01864	33.83	<.001
Cub	1	0.92307	0.92307	30.66	<.001
Deviations	2	0.21847	0.10923	3.63	0.032
inoculum	3	33.32588	11.10863	368.97	<.001
Lin	1	23.18076	23.18076	769.94	<.001
Deviations	2	10.14512	5.07256	168.48	<.001
Zn.inoculum	15	1.18216	0.07881	2.62	0.004
Lin.Lin	1	0.00005	0.00005	0.00	0.967
Quad.Lin	1	0.01243	0.01243	0.41	0.523
Lin.	2	0.20749	0.10375	3.45	0.037
Cub.Lin	1	0.20933	0.20933	6.95	0.010
Quad.Dev	2	0.48178	0.24089	8.00	<.001
Deviations	8	0.27107	0.03388	1.13	0.357
Residual	69	2.07740	0.03011		
Total	95	39.38019			

***** Tables of contrasts *****

***** rep.plot stratum *****

*** Zn contrasts ***

Lin -0.0261 s.e. 0.00805 ss.div. 464.

Quad 0.133 s.e. 0.0229 ss.div. 57.4

Cub -1.62 s.e. 0.292 ss.div. 0.353

Deviations e.s.e. 0.0434 ss.div. 16.0

Zn	0.00	0.01	0.04	0.10	0.40	6.00
	-0.074	0.043	0.069	-0.039	0.002	0.000

*** inoculum contrasts ***

Lin 0.1661 s.e. 0.00599 ss.div. 840.

Deviations e.s.e. 0.0354 ss.div. 24.0

inoculum	0.00	2.00	4.00	8.00
	-0.411	0.401	0.220	-0.210

*** Zn.inoculum contrasts ***

Lin.Lin 0.0001 s.e. 0.00272 ss.div. 4063.

Quad.Lin 0.0050 s.e. 0.00774 ss.div. 502.

Lin.Dev e.s.e. 0.0161 ss.div. 116.

inoculum	0.00	2.00	4.00	8.00
	0.026	-0.030	-0.009	0.012

Cub.Lin -0.260 s.e. 0.0988 ss.div. 3.09

Quad.Dev e.s.e. 0.0458 ss.div. 14.4

inoculum	0.00	2.00	4.00	8.00
	-0.116	0.112	0.063	-0.060

Deviations e.s.e. 0.0868 ss.div. 4.00

Zn inoculum	0	2	4	8
0.00	0.027	0.023	-0.033	-0.017
0.01	-0.073	0.112	0.017	-0.056
0.04	-0.053	-0.056	0.020	0.089
0.10	0.123	-0.112	-0.001	-0.010
0.40	-0.025	0.032	-0.002	-0.005
6.00	0.000	0.000	0.000	0.000

Analysis of variance: Without noninoculated treatment, log transformation

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.42423	0.14141		
rep.plot stratum					
Zn	5	3.30210	0.66042	17.09	<.001
inoculum	2	1.80658	0.90329	23.37	<.001
Zn.inoculum	10	0.35663	0.03566	0.92	0.520
Residual	51	1.97135	0.03865		
Total	71	7.86089			

***** Tables of means *****

Grand mean 1.323

Zn	0.00	0.01	0.04	0.10	0.40	6.00
	1.515	1.608	1.469	1.051	1.125	1.171

inoculum	0	2	4	8
		1.145	1.296	1.530

Zn inoculum	0.00	2.00	4.00	8.00
0.00		1.382	1.464	1.698
0.01		1.543	1.577	1.703
0.04		1.277	1.455	1.674
0.10		0.906	1.085	1.163
0.40		0.862	1.046	1.468
6.00		0.896	1.146	1.472

*** Standard errors of differences of means ***

Table	Zn	inoculum	Zn X inoculum
rep.	12	24	4
s.e.d.	0.0803	0.0568	0.1390

Analysis of variance and polynomial contrast, excluding noninoculated treatments.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
rep stratum	3	0.42423	0.14141		
rep.plot stratum					
Zn	5	3.30210	0.66042	17.09	<.001
Lin	1	0.42187	0.42187	10.91	0.002
Quad	1	1.35819	1.35819	35.14	<.001
Cub	1	1.23076	1.23076	31.84	<.001
Deviations	2	0.29129	0.14564	3.77	0.030
inoculum	2	1.80658	0.90329	23.37	<.001
Lin	1	1.79857	1.79857	46.53	<.001
Deviations	1	0.00801	0.00801	0.21	0.651
Zn.inoculum	10	0.35663	0.03566	0.92	0.520
Lin.Lin	1	0.09834	0.09834	2.54	0.117
Quad.Lin	1	0.15401	0.15401	3.98	0.051
Lin.Dev	1	0.00373	0.00373	0.10	0.757
Cub.Lin	1	0.01158	0.01158	0.30	0.586
Quad.Dev	1	0.00066	0.00066	0.02	0.897
Deviations	5	0.08831	0.01766	0.46	0.806
Residual	51	1.97135	0.03865		
Total	71	7.86089			

***** Tables of contrasts *****

*** Zn contrasts ***

Lin -0.035 s.e. 0.0105 ss.div. 348.

Quad 0.178 s.e. 0.0300 ss.div. 43.1

Cub -2.16 s.e. 0.382 ss.div. 0.265

Deviations e.s.e. 0.0568 ss.div. 12.0

Zn	0.00	0.01	0.04	0.10	0.40	6.00
	-0.099	0.057	0.092	-0.052	0.002	0.000

*** inoculum contrasts ***

Lin 0.0634 s.e. 0.00929 ss.div. 448.

Deviations e.s.e. 0.0401 ss.div. 24.0

inoculum	0.00	2.00	4.00	8.00
		-0.010	0.015	-0.005

*** Zn.inoculum contrasts ***

Lin.Lin 0.0067 s.e. 0.00422 ss.div. 2167.

Quad.Lin -0.024 s.e. 0.0120 ss.div. 268.

Lin.Dev e.s.e. 0.0182 ss.div. 116.

inoculum	0	2	4	8
		-0.003	0.005	-0.002

Cub.Lin -0.08 s.e. 0.153 ss.div. 1.65

Quad.Dev e.s.e. 0.0519 ss.div. 14.4

inoculum	0	2	4	8
		-0.004	0.005	-0.002

Deviations e.s.e. 0.0983 ss.div. 4.00

Zn inoculum	0.00	2.00	4.00	8.00
0.00		0.001	-0.032	0.031
0.01		0.068	-0.012	-0.056
0.04		-0.063	0.005	0.058
0.10		-0.015	0.054	-0.039
0.40		0.009	-0.014	0.006
6.00		0.000	0.000	0.000

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