Role of Zn Nutritional Status on Infection of *Medicago* Species by *Rhizoctonia solani*

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Abstract

A decline in the quality of medic pastures has been observed across Australia which may be due in part to a causal relationship between Zn deficiency and increasing severity of Rhizoctonia solani infection. Zinc deficiency is widespread in Australia and with the use of minimum till farming practices, the incidence of infection by R. solani has increased on Zn-deficient soil. The primary aim of this thesis was to determine if Zn-deficient medic are more sensitive to infection by R. solani.

To determine if a relationship between Zn nutrition and infection by R. solani existed in medic, a field survey was performed in 1994. The paddock was infected with R. solani and Zn had been previously applied with varying rates of application. Since Zn fertiliser has a residual effect in the soil, and R. solani was present, this paddock was ideal for a survey. The concentration of Zn in the medic shoots was inversely related to the severity of disease.

To further understand the effects of R. solani on the nutritional status of medic, Medicago truncatula genotype Caliph was grown in a non-nutrient-limiting controlled environment with three levels of R. solani in the soil (0, 1 and 8 propagules/kg soil). Plants were harvested at 14, 28, 42 and 56 days. With increasing levels of R. solani, to change occurred in dry matter accumulation at any harvest time, no was there any change in the concentrations of nine out of ten elements analysed in the shoot. Manganese was the only element to become lower in the shoot with increasing levels of R. solani. At this level of R. solani, the pruning effect of R. solani does not have a significant effect on the capacity for maintaining adequate levels of nutrients in the plant, when grown in non-nutrient-limiting conditions.

To determine the effect of R. solani (A08) on the growth of medic with declining levels of Zn, a factorial experiment with five levels of Zn fertiliser (0, 0.05, 0.1, 0.3 and 0.9 mg Zn/kg)
and six levels of *R. solani* inoculum (0, 1, 2, 4, 8 and 16 propagules/kg soil) was performed. This experiment demonstrated for the first time an increase in tolerance to *R. solani* with Zn application in medic. Although the disease score on the root system declined with increasing level Zn fertiliser, the number of infection points on the root system was higher, indicating the presence of more pathogen on the roots of medic supplied with Zn.

A subsequent factorial experiment of four inoculum levels (0, 1, 2, 4, and 32 propagules/kg soil) and two Zn levels (0 and 0.9 mg Zn/kg) was conducted to investigate the effect of infection by *R. solani* (AG8) on the root system in more detail. Compared with Zn-deficient medic, medic fertilised with Zn not only had a higher number of infection points on the root system as a result of infection by *R. solani*, but more *R. solani* DNA was extracted from the root systems. However, the addition of Zn allowed medic to reach maximum yield potential despite the heavier infection with *R. solani*. Medic also maintained a stable concentration in the shoot of all analysed nutrients when Zn was applied, while Zn-deficient plants had a reduction in nutrient concentration with severe levels of *R. solani*. In contrast to this, Zn-deficient plants in the presence of a low amount of *R. solani* (1 propagule/kg soil) increased concentration of most nutrients in the shoot. This occurred in parallel with an increase in fine root length and surface area, which was not observed in medic supplied with Zn. Overall, the results indicate Zn application does not directly inhibit the fungus or reduce its pathogenicity but does strongly increase root growth. The net result is that Zn increases tolerance to the pruning effects of the fungus, and this is most likely due to compensation in nutrient and water uptake from a greater mass of uninfected root axes.

A field experiment was performed to determine if residual levels of Zn applied three years prior to sampling were beneficial to a medic pasture. Zinc fertiliser was applied in the surface soil (0-0.05m) and in the sub-soil (0.05-0.40m) in combination with N and P fertiliser in 1993. Calliph medic was sampled in 1996 with a response to Zn fertiliser observed in concentration of shoot and in seed. A higher concentration of Zn occurred in seed when Zn was applied to the top soil (as granulated fertiliser), compared to the sub-soil (as a liquid
solution). This is most probably due to the slow-release nature of granulated Zn. Although a response to Zn occurred, all plants were deficient in Zn. Zinc fertilizer should therefore be applied at higher concentrations in the first instance or applied more frequently.

Field and pot experiments were performed to determine if Zn-efficient medic could be identified. Twelve genotypes were sown in the field on Zn-deficient soil with two levels of Zn (0 and 8 kg Zn/ha), while 19 genotypes were planted in a controlled environment with four levels of applied Zn (0, 0.1, 0.3 and 0.9 mg Zn/kg soil). Overall, four genotypes from M. truncata were identified as Zn-efficient (Rivoli, SA20248, SA20278 and SA20521). Rivoli was grown in both experiments and ranked Zn-efficient in each. Although Rivoli did not have a high yield potential, yield potential was reached at the lowest level of Zn in both the field and pot experiments. The remaining three Zn-efficient genotypes were high yielding under Zn deficiency but maximum yield occurred under conditions of non-limiting Zn only. The effect of seed size was also studied in the field on two of genotypes planted using small and large seed. Although differences in seed size influenced final yield, the Zn efficiency ranking was not affected.

The final experiment was designed to determine if Zn-efficient genotypes have a higher tolerance to R. solani under Zn-deficient conditions than Zn-inefficient genotypes. Five genotypes, two Zn-efficient (SA20378 and SA20521), one moderately Zn-efficient (Caliph) and two Zn-inefficient (SA18286 and Z1064) were grown under Zn-deficient conditions in a controlled environment, and seed collected. Since the seed was low in Zn, Mg, Na, K and P it had a lower nutritional value than seed used in previous experiments. This seed was used in the subsequent experiment. A factorial experiment with five genotypes, four levels of R. solani (0, 1, 8 and 32 propagules/kg soil) and two Zn applications (0 and 0.9 mg Zn/kg) was conducted. All genotypes generally had a lower dry matter accumulation with increasing levels of R. solani at both Zn treatments. Unlike the remaining genotypes, Zn-efficient genotypes maintained a stable concentration of all nutrients in the shoot under Zn-deficient conditions, indicating a higher tolerance to the pathogen. The use of low quality seed resulted
in a reduction in tolerance to *R. solani* and a lower Zn-efficiency ranking in all genotypes.

This thesis identifies a strong relationship between Zn nutrition of medics and severity of disease by *R. solani* (AG8) which may therefore be a major factor contributing to the current decline in medic pastures observed in Australia. The effect of seed nutritional value had a profound impact on the growth of medics and may also be a key factor in poor medic growth.