Allelopathic interference of annual ryegrass (*Lolium rigidum*) on lucerne nodulation

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Summary Laboratory and glasshouse experiments were undertaken to evaluate the allelopathic effects of annual ryegrass (*Lolium rigidum* Gaudin, ARG) root exudates against growth and nodulation of lucerne genotypes. This paper reports the outcomes of the nodulation investigation. The impact of ARG on lucerne nodulation was evaluated using SARDI Five and Titan 9 lucerne cultivars (in both laboratory and glasshouse) at 6 rhizobium (*Sinorhizobium* spp.) concentrations (0, 0.6, 1.5×10, 1.5×10^2, 1.5×10^3, 1.5×10^4 colony forming units mL^{-1}). At the highest rhizobium concentration, a fivefold reduction in nodule number was observed in the laboratory when lucerne genotypes were grown with ARG. Increasing annual ryegrass density also reduced nodulation under glasshouse conditions. The variability identified within and between lucerne populations suggests that there is scope to reduce allelopathic interference of ARG on seedling lucerne growth and improve the compatibility of these species in pasture mixtures at establishment.

Keywords Allelopathy, annual ryegrass, inhibition, lucerne, nodulation.

INTRODUCTION

Plant communities evolve as a result of the interactions of individual plants with their neighbours. This will be influenced by characteristics such as relative competitiveness and chemical interference. The chemical interference, known as allelopathy, refers to a mechanism whereby a plant releases, into the surrounding environment, bioactive metabolites that negatively affect the germination, establishment and growth of other plants growing in the vicinity (Anaya 1999, Asaduzzaman *et al*. 2014b). This interaction occurs in both directions with the outcome depending on relative chemical potencies and plant tolerances (Moore *et al*. 2015). The effect is usually on root growth and, in legumes, it might be anticipated that nodulation might be compromised.

Leaf leachates and root exudates of some weeds have been shown to affect nodulation adversely in white clover (*Trifolium repens* L.), red kidney beans (*Phaseolus vulgaris* L.) and Korean lespedeza (*Lespedeza stipulacea* Maxim.) (Rice 1968, 1971, Weston and Putnam 1985). Annual ryegrass is the most common weed in temperate crop production in Australia and is a regular companion plant in temperate pastures. It is well reported to have allelopathic impacts on crops (e.g. Wu *et al.* 2000, Asadazzaman 2014a). However, there are no recent studies that have assessed the allelopathic potential from root exudates of annual ryegrass (ARG, *Lolium rigidum* Gaudin) on lucerne as a companion plant in a pasture sward.

The aim of this study was to evaluate the allelopathic capability of ARG against lucerne genotypes to investigate whether such inhibition might extend to root nodulation in the legume.

MATERIALS AND METHODS

Laboratory experiment A modification of Gibson's tube culture technique (Gibson 1963) was used for growing the lucerne plants. Agar (25 mL of 0.5% nutrient free water agar) was added to each glass tube (150 × 25 mm). The tubes were plugged with foam bungs. After autoclaving, the tubes were sloped so that the agar extended to the top of the tube. Pre-germinated seeds of ARG at the density of four seeds per tube were aseptically sown on the middle position of the agar surface in one row. The tubes were then kept in a growth incubator. After seven days, pre-germinated seeds of lucerne, cultivars SARDI Five and Titan 9, were introduced at a single seed per tube. Six different

rhizobium concentrations (0, 0.6, 1.5×10, 1.5×10^2, 1.5×10^3, 1.5×10^4 colony forming units mL^{-1}) at 1 mL per tube were added to the agar surface of the tube which was then placed back in the growth chamber for seven days. 'The plate count method' was used for rhizobium enumeration (Vincent 1970). After 7 days of growing in concert, the ARG seedlings were removed and 2 mL of nutrient solution were included per glass tube and incubated for another four weeks in the growth incubator. Half strength Jensen's (1942) nutrient solution was used. Nodule numbers were counted after four weeks. The control treatment comprised growth of lucerne seedlings without the presence of ARG. The tubes were arranged in a randomised complete block design along with eight replications under controlled condition.

Glasshouse experiment Two lucerne genotypes, cultivars SARDI Five and Titan 9, were used as test species in this experiment based on previous findings. Pre-germinated seeds of annual ryegrass were sown in cylindrical PVC pots (90 mm diameter × 210 mm height). Each pot was filled with 1.6 kg (oven dry basis) brickie sand. The sand was pasteurised with steam. All pots were maintained at a water content of approximately 80% of field capacity by daily weighing and watering with deionised water. ARG densities used were 0, 5, 10 and 20 seedlings and there were four circles of these densities per pot. After the growth of ARG seedlings for a week, single pre-germinated seed of each of two lucerne genotypes were sown in the middle of the each circle. Pots were inoculated with six different rhizobium concentrations (0, 0.6, 1.5×10, 1.5×10^2, 1.5×10^3, 1.5×10^4 colony forming units mL^{-1}) at 1 mL per lucerne plant. After 7 days of growing in concert, the ARG seedlings were uprooted and N-free nutrient solution was applied. The growth of lucerne seedlings without ARG was the control. Plants were grown under natural lighting in the glasshouse from April to June in Wagga Wagga, New South Wales. Temperatures were regulated between 25°C (max) and 15°C (min). Three replicates were used and pots were arranged in a randomised block design. Lucerne plants were harvested four weeks after removal of ARG. Shoots were separated from roots. Roots were washed from the sand and the root data were assessed by scanning and image analysis using WinRhizo software (Regent Instruments, Montreal). Nodules were counted for accuracy using scan images. In addition, images of fully elongated root hairs were taken using a Nikon SMZ 25 Stereo microscope fitted with a Nikon DS-Fi2 camera. Ten images were captured per plant and root hair lengths of 10 root hairs per image were measured using ImageJ software (Rasband 1997–2014). Root and shoot dry mass were determined after drying at 70°C.

Data were subjected to analysis of variance using Genstat v17 (VSN International, Hemel Hempstead, United Kingdom). The model assumptions used were: i) the residuals are normally distributed; ii) the residuals are independent; iii) the residuals have a mean of zero; iv) the residuals have constant variance; v) the treatment groups are normally distributed; and vi) homogeneity of variances of the data within factor levels. These model assumptions were checked and met in every case. Where significant treatment differences were found, a Tukey's pairwise multiple comparison test (Tukey 1949) was used.

RESULTS

Laboratory experiment Root nodule numbers of SARDI Five and Titan 9 lucerne cultivars were reduced ($P<0.01$) by the root exudates of ARG relative to the control (Figure 1). Nodule numbers of Sardi 5 were reduced sixfold and Titan 9 eightfold relative to the controls due to the presence of ARG.

Glasshouse experiment The main effects of lucerne genotype, rhizobium concentration and their interactions on root hair length were not significant. However annual ryegrass density had a significant ($P<0.05$)

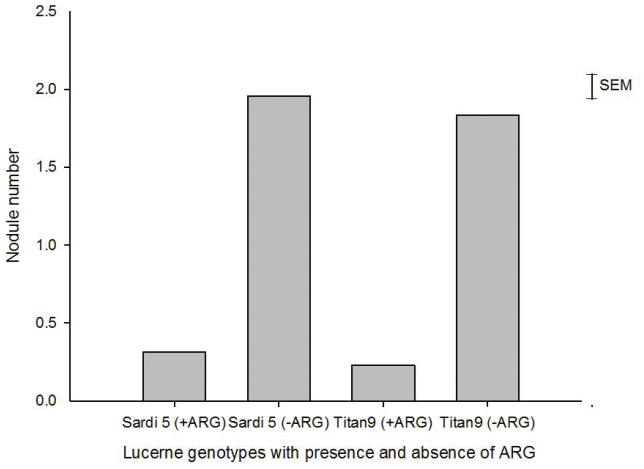

Figure 1. Impact of annual ryegrass presence of nodulation number on roots of lucerne grown in tube culture.

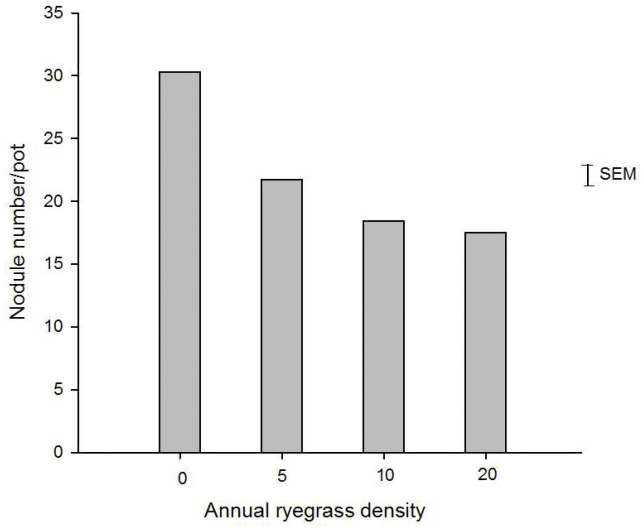

Figure 2. The effect of annual ryegrass seedling densities on lucerne nodule numbers per pot.

effect on lucerne root hair length (data not shown). The lowest root hair lengths recorded were 436 µm and 433 µm at the densities of 10 and 20 seedlings per beaker respectively compared with control (475 µm). ARG density significantly ($P<0.01$) reduced lucerne nodule number (Figure 2) compared with lucerne growing alone. At the highest ARG density, nodule density was approximately halved at 17 nodules per pot relative to 30 nodules per pot for the control.

DISCUSSION

Nodule development involves interaction between rhizobia and legume root hairs (Schultze and Kondorosi 1998, Limpens and Bisseling 2003). The development of a root nodule is under the control of nodulation (nod) genes (Eckardt 2006). Legume roots secrete flavonoids which perform as signals to activate the expression of nod genes resulting in the production of rhizobial nod factors that cause root cortical cells to breakdown and develop into nodules (Schultze and Kondorosi 1998, Spaink 2000). Both laboratory and glasshouse experiments have shown that root exudates from ARG affect the development of nodules in lucerne genotypes. The reduction in root hair length was around the order of 10% whereas nodule number reduction was nearly 50% due to the association with ARG allelochemicals, suggesting that root structures have been compromised. This finding has implications for productivity in the field where effective nodulation is a fundamental to legume-based pastures and pulse crops. Related work (H. Zubair, unpubl. data) has shown that there is variable tolerance in lucerne genotypes in their susceptibility to root growth inhibition due to ARG allelopathy. It may also be a factor in nodulation susceptibility as well, although genotype screening has not been done. This work also raises the question of species and cultivar compatibility in mixed pasture swards and the need to select companions which minimise the incompatibility.

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