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Interactions between lucerne, rhizobia and mycorrhizas under different levels of N and P in the glasshouse and field

A thesis

submitted in partial fulfillment

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by

Quang Ngoc Mai

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Abstract of a Thesis submitted in partial fulfillment of the requirement for the Degree of Master of Agricultural Science

Interactions between lucerne, rhizobia & mycorrhizas under different levels of N and P in the glasshouse and field

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Use of nitrogen (N) fixing legumes and mycorrhizal inoculants have the potential to reduce the effects of limiting soil N and phosphorus (P) levels in agricultural soils. In New Zealand, lucerne is used for direct grazing and hay making and it is recommended that rhizobia (*Ensifer meliloti*) and P be added to this crop for maximum production. The specific objectives of this thesis were to test under different soil N and P availability: 1) The effectiveness of *Ensifer meliloti* rhizobial inoculum and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand on growth of lucerne. 2) The effectiveness of commercial vesicular arbuscular mycorrhizas on growth of lucerne. 3) The interaction between rhizobial and mycorrhizal inoculant on growth of lucerne.

In pots, *Ensifer meliloti* increased, but *Rhizobium* sp. isolated from New Zealand soils decreased, lucerne total plant dry matter. It seems likely that under field conditions, competition between *Ensifer meliloti* in the inoculum and less effective indigenous soil rhizobial strains for nodulation can reduce the efficiency of the inoculum. Addition of N and P with *Ensifer meliloti* both increased lucerne total plant dry matter but shoot crude protein, dry matter digestibility and metabolisable energy were not affected. Plants relying solely on N_2 fixation had around 90% total dry matter of plants on optimum soil N indicating that there is little benefits of adding N to lucerne if it is adequately nodulated. Addition of P is required in low P soils to achieve high production.

Under field conditions, addition of mycorrhizal inoculum or 16 kg P/ha gave similar increased dry matter yield but addition of rhizobial inoculant did not affect yield. It seems likely that there were already high populations of rhizobia in the soil before rhizobial inoculation as plants sampled from uninoculated plots showed substantial nodulation. Thus, the effect of additional rhizobia was negated.

Overall, similar yield increases of lucerne with mycorrhizas and added 16 kg P/ha is an important finding. The potential of mycorrhizas as a mechanism to reduce P inputs into lucerne crops warrants further testing under different soils and agricultural systems in New Zealand.

Keywords: lucerne, rhizobia, mycorrhiza, nitrogen, phosphorus, growth, *Ensifer meliloti*, *Glomus mosseae*.

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ABBREVIATIONS

Abbreviation	Description	Units
CP	Crude protein	%
DM	Dry matter	Kg/ha or g/m^2
DMD	Dry matter digestability	%
ME	Metabolisable energy	MJ kg^{-1} DM or GJ $ha^{-1}yr^{-1}$
N	Nitrogen	Kg/ha
P	Phosphorus	Kg/ha
R^2	Coefficient of determination	-

Chapter 1. GENERAL INTRODUCTION

1.1 Plant/crop requirements for growth

For plants to grow and develop, they need light, carbon dioxide (CO_2), water (H_2O), oxygen (O_2), a range of macronutrients and micronutrients, temperature within a specific range and space (Fitter and Hay, 2002; Engels *et al.*, 2012; Kirkby, 2012). Light along with CO_2 and H_2O are required for the process of photosynthesis which can be summarised as:

$$CO_2 + H_2O \xrightarrow[\text{Chlorophyll a}]{\text{light}} CH_2O + O_2$$

Chlorophyll a is the major photosynthetic pigment in crop plants and it alone can convert light into chemical energy. The other main photosynthetic pigments are chlorophyll b and carotenoids. These pigments absorb light. Light can also be involved in photoperiod (day length/night length) (Hay and Porter, 2006) and phototropic (directional response of growth to light) responses in plants (Monteith, 1981; Mattera *et al.*, 2013).

In the process of photosynthesis, CO_2 is taken up directly from the atmosphere via stomata in leaves and diffuses into the chloroplast. Water is taken up from the soil by roots. It then, in turn, enters the root, stem and leaf xylem. Some water is taken up by cells then some diffuses into the chloroplast. Most of the water taken up by roots is lost to the atmosphere via stomata, small pores on the surface of leaves, through the process of transpiration (Fitter and Hay, 2002; Hay and Porter, 2006). There can be thousands of stomata cm^{-2} usually on the underside of leaves. Stomatal aperture changes relative to water availability; it decreases under water stress. This change results in less H_2O loss, but it also results in reduced CO_2 uptake and fixation.

Greater than 95% of all plants are 'C_3' plants. These plants produce a 3 carbon (C) compound on CO_2 fixation (Still *et al.*, 2003; Sage *et al.*, 2012). Here CO_2 reacts with 5C ribulose bisphosphate in the presence of the enzyme ribulose bisphosphate carboxylase (rubisco) to produce two × 3C phosphoglycerate. All the main temperate cereals (e.g. wheat (*Trifolium aestivum*), barley (*Hordeum vulgare*)), grasses (e.g. perennial ryegrass (*Lolium perenne*), cocksfoot grass (*Dactylis glomerata*)), grain legumes (e.g. pea (*Pisum*

sativum), lentil (*Lens culinaris*)) and pasture legumes (white clover (*Trifolium repens*)) and lucerne (*Medicago sativa*) (the plant species studied in this thesis) are C_3 plants. For lucerne, sucrose is the main product of photosynthesis transported in the phloem but starch is the most important storage form of carbohydrate in roots (Hall, 1987; Heichel *et al.*, 1988).

Less than 5% of plants are 'C_4' plants. These plants produce a 4C compound on CO_2 fixation. Here CO_2 reacts with the 3C phosphoenolpyruvate (PEP) in the presence of the enzyme PEP carboxylase to produce a 4C organic acid, commonly malate (Sinclair and Horie, 1989; Sage *et al.*, 1999; 2012). This reaction takes place in the leaf mesophyll cells. The organic acid is then transported to the bundle sheath cells where CO_2 is released and fixed via rubisco as for C_3 plants. C_4 plants are generally tropical grasses: examples are maize (*Zea mays*), sugarcane (*Saccharum officinarum*) and kikuyu grass (*Pennisetum clandestinum*).

Generally, water use efficiency (H_2O used per CO_2 fixed) is greater with C_4 plants than C_3 plants (Sage *et al.*, 2012; Busch *et al.*, 2013). This is because PEP carboxylase has a greater affinity for CO_2 in comparison with that for rubisco and thus C_4 plants can have a high photosynthetic rate with a small stomatal aperture/high stomatal resistance. C_3 plants show very low photosynthetic rates with high stomatal resistance.

Water is required for plant processes in addition to photosynthesis. It is needed for cell expansion and to maintain cell turgor and is the general solvent for all the plant reactions (Slatyer, 1967; Ludlow and Muchow, 1990; Farmer and Browse, 2013). It is also the means by which substances are transported long distances in the plant via the xylem and phloem (van Bel, 1990; White, 2012). Loss of water from leaves is important in evaporative cooling of leaves on hot days (de Wit, 1958; Cooper *et al.*, 1983).

Oxygen is a product of photosynthesis but it is also required for the process of respiration which provides the plant with energy required for plant function (Sage, 1994; Hohmann-Marriott and Blankenship, 2011; Sage *et al.*, 2012; Busch *et al.*, 2013; Denton *et al.*, 2013). The overall reaction of respiration is:

$$CH_2O + O_2 \xrightarrow[\text{energy}]{} CO_2 + H_2O$$

There are six macronutrients required by plants, these are Nitrogen (N), Phosphorus (P), potassium (K), Magnesium (Mg), Sulphur (S) and Calcium (Ca) (Broyer and Stout, 1959; Hawkesford *et al.*, 2012). All of these nutrients are essential elements, elements that plants cannot complete their lifecycle without. Macronutrients are generally taken up as ions from the soil, N as nitrate (NO_3^-) and ammonium (NH_4^+), P as phosphate (PO_4^{3-}), S as sulphate (SO_4^{2-}), K as K^+, Mg as Mg^{2+} and Calcium as Ca^{2+} (Hossner, 2008). This thesis focuses on N and P nutrition of lucerne and this is considered in more detail in Chapter 2.

There are also at least nine essential element micronutrients. There are Iron (Fe), Boron (B), Manganese (Mn), Copper (Cu), Zinc (Zn), Molybdenum (Mo), Nickel (Ni), Cobalt (Co), and Chlorine (Cl). In addition, some plants require Sodium (Na), Selenium (Se) and Silicon (Si). Overall, the bulk of plant dry weight is in the form of C (usually 35-45%), O (35-45%), H (~6%), N (1-6%) and K (1-6%) (Chesworth, 2008; Broadley *et al.*, 2012; Engels *et al.*, 2012).

Plants require temperature within a certain range. This temperature range depends on species and for crop plants, cultivar. Plants also require space to grow and develop. At the crop level, individual plant growth is dependent on crop density (plant population) and weed density (Willey and Heath, 1969; Ford, 1975; Hay and Porter, 2006; Taiz and Zeiger, 2010). Weeds are plants so can compete with crops for all plant requirements (Berti *et al.*, 2008; Robert, 2008).

1.2 Factors limiting crop growth

All factors required for crop growth can limit crop growth if not in adequate supply. In relation to nutrient availability and potential crop growth, worldwide, soils are more commonly deficient in N than any other nutrient element (Sprent, 1990; Hay and Porter, 2006; 2009). This is followed by P and then K deficiency (Robson *et al.*, 1981; Raven *et al.*, 2005; Edmeades *et al.*, 2010). Low water availability is also a major restriction to crop growth in many parts of the world (Ludlow and Muchow, 1990).

Generally, in intensive agricultural systems, N and P limitations to crop growth are overcome by adding inorganic nutrient fertilisers. Similarly, water limitations on crop growth are countered by irrigation. However, use of inorganic fertilisers and irrigation have been linked to environmental problems (Monaghan *et al.*, 2007). In relation to synthetic N fertiliser, in most intensive agricultural systems over 50% of the N applied to the crop is not used by the plant (Thompson *et al.*, 2007). Much of this surplus N is liable to be lost to the aqueous and atmospheric environments where it can become a serious pollutant (Bussell *et al.*, 2006). The production and use of synthetic N fertiliser has contributed to the increased emission of greenhouse gases to the atmosphere, a decreased biodiversity within and outside the agricultural systems and eutrophication of fresh waters, estuaries, coastal waters and nutrient poor land habitats (Saggar *et al.*, 2004; Cameron *et al.*, 2013; Moir *et al.*, 2013). Loss of nitrate leads to loss of positive charged ions such as K^+, Mg^{2+}, and Ca^{2+} (Andrews *et al.*, 2009b; Di and Cameron, 2012). Increased leaching of P, associated with increased application of P fertiliser has also contributed to decreased biodiversity within and outside agricultural systems and eutrophication of water and land habitats (Hall *et al.*, 2003; Wang *et al.*, 2004; Cameron *et al.*, 2013).

Irrigation can result in increased leaching of nutrients into waterways (Clark *et al.*, 2007). In addition, increased salinity due to continual irrigation is an increasing problem in many countries (Condron *et al.*, 2000; Qadir and Oster, 2004). These environmental problems associated with high use of N and P fertiliser and irrigation have led to the search for alternative strategies to combat limiting N, P and water availability on crop growth (Andrews *et al.*, 2003; 2011a; 2011b).

1.3 N_2 fixing legumes and mycorrhizas

Alternative strategies are being sought to the application of synthetic N fertiliser as a means of combating limiting soil N levels in agricultural soils. One alternative method is to use a N_2 fixing legume as, for example, a seed crop, a green manure, a forage crop or as the main N input into a grass pasture by growing it in association with the grass (Andrews *et al.*, 2009a; 2010). Use of legume N_2 fixation instead of synthetic N fertiliser would avoid greenhouse gas emissions resulting from N fertiliser production. Also, N

input into a system using N_2 fixing legumes is cheaper than use of N fertiliser. However, at the crop level, N loss to the external environment relative to N accumulated by N_2 fixing legumes is very variable. In the case of non-fertilised grain legumes for which the harvested part is taken off-site, and relatively little residual N is left in the soil, loss of N per unit of N accumulated is likely to be very low. In contrast, N losses relative to N accumulated are likely to be similar for a grass/legume pasture and a N-fertilised grass pasture of similar productivity (Andrews *et al.*, 2007; 2011a). However, the P requirement of legumes is as great if not greater than that for non-legume crops and this also must be considered (Andrews *et al.*, 2007).

There is no microorganism that can add P to agricultural systems in the way that legumes add N, but mycorrhizas can increase the availability of P to crops (Andrews *et al.*, 2010). For example, some rhizobacteria can increase P availability via P solubilisation. More important, is the mycorrhizal uptake of water and nutrients. Mycorrhizas are fungal associations with plants. Mycorrhizal symbioses can occur with around 90% of plants and supply the associated plant with additional P in particular, but also N and other nutrients and water under certain conditions (Hodge and Andrews, 2004; Andrews *et al.*, 2010). Increased nutrient uptake with mycorrhizas is related to the smaller diameter, greater degree of branching and greater longevity of their hyphae in comparison with root hairs, and their greater secretion of low molecular weight metabolites and of enzymes making inorganic P and soluble low molecular mass organic N compounds available (Lambers *et al.*, 2008; Raven, 2010; Smith *et al.*, 2010).

1.4 Lucerne (*Medicago sativa*)

Lucerne (alfalfa) is a perennial legume that plays an important role in world agriculture. It is the world's most important temperate forage crop and the fourth most important crop in the United States of America (Small, 2011). It has been called the 'Queen of forage crops' (Burton, 1972). Lucerne is thought to have been cultivated for several thousand years (Michaud *et al.*, 1988). Cultivation of lucerne appears to have originated in the Mediterranean and Persian regions and eventually spread to Europe, North and South America, Asia and Oceania (Michaud *et al.*, 1988).

Lucerne contributes around 1 trillion dollar (US) to the world economy annually via its use in many crop products including forage, fodder, hay, seed, honey, sprouts and even in pharmaceutical and industrial enzymes (Small, 2011). Depending on environmental conditions and variety, lucerne can persist for 5 to 20 years. Lucerne is cultivated in over 32 million hectares worldwide (Michaud *et al.*, 1988; Irwin *et al.*, 2001).

Lucerne is a legume and can form symbiotic associations with rhizobia in root nodules that can fix atmospheric N_2 (Burton, 1972; Sprent, 2001, 2009). The most common rhizobial species associated with lucerne is *Ensifer meliloti* (Sprent, 2009). This ability to fix N_2, can result in very high production of lucerne in low N soils as long as other factors do not severely limit growth. Also, lucerne has a large and deep taproot that can extract soil water and nutrients from deep layers (Brown *et al.*, 2005a; Moot *et al.*, 2008; 2012), and thus can survive longer and produce higher dry matter and protein yields in dry conditions in comparison with many other forage legumes (Boller and Heichel, 1983; Fick *et al.*, 1988; Moot *et al.*, 2008). In addition, lucerne forms mycorrhizal associations with specific fungal species (Ardakani *et al.*, 2009a; 2009b).

Lucerne has been cultivated in New Zealand since the early 20th century. Production reached a peak of 220,000 ha in 1978 and decreased to 101,200 ha in 1984, with nearly 80% of total areas on the South Island, due to the impacts of pests, diseases, unfavourable weather, and inappropriate grazing management (Dunbier *et al.*, 1982; Douglas, 1986). In New Zealand, lucerne is used for direct grazing (~36%) and hay making (Douglas, 1986). Over the past 30 years, New Zealand experienced serious drought especially in 1985, 1988, and 1996 on the east coast and hill country (Avery *et al.*, 2008). The South Island hill country typically has a short water limited growing season, and greater use has been made of lucerne crops in these areas over the past 10 years (Moot *et al.*, 2012). Cultivars have been developed such as 'Marlborough', 'Oranga', 'Ontario', and 'Kaituna' with greater pest and disease resistance (White, 1982; Wynn-Williams, 1982; Brown and Green, 2003; Moot *et al.*, 2003).

1.5 Objectives of thesis

The specific objectives of this thesis were to test under different soil N and P availability:

Objective 1. The effectiveness of *Ensifer meliloti* and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand on growth of lucerne (Chapters 3, 4).

Objective 2. The effectiveness of commercial vesicular arbuscular mycorrhizas on growth of lucerne (Chapter 4).

Objective 3. The interaction between rhizobial and mycorrhizal inoculant on growth of lucerne (Chapter 4).

Objective 4. On finding that *Ensifer meliloti* but not the *Rhizobium* sp. strains gave increased growth a fourth objective was set which determined how growth of lucerne relying primarily on N fixation matched that of plant at optional soil N (Chapter 3).

Finally, in Chapter 5 the findings of the experiments and the potential applications for the knowledge gained are discussed.

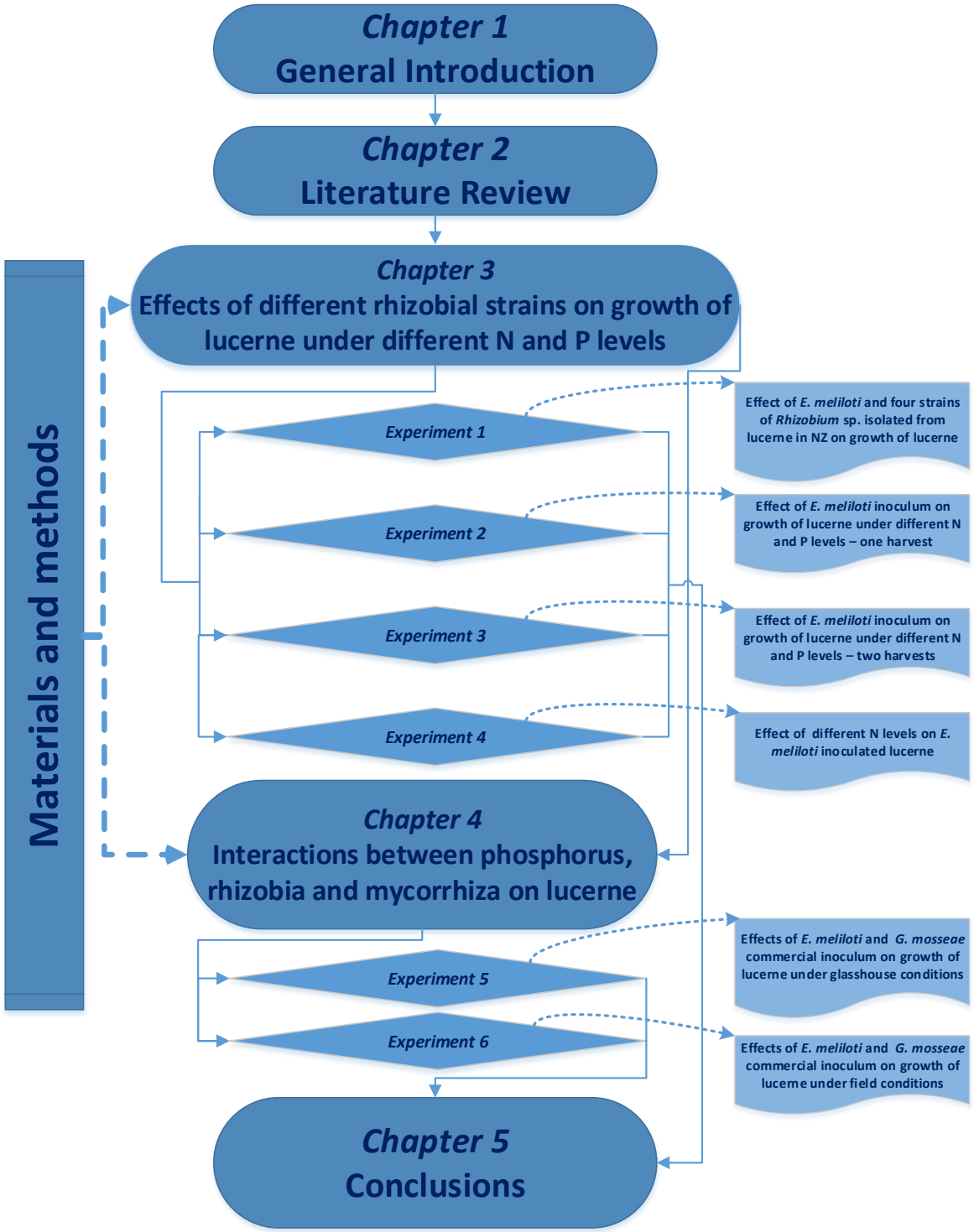

Figure 1.1. Outline of thesis structure and main topics dealt with in each Results chapter.

Chapter 2. REVIEW OF THE LITERATURE

2.1 Nitrogen, phosphorus and water limitations on crop growth

Worldwide, soils are more commonly deficient in N than any other nutrient element. This is followed by P, and low water availability is also a major restriction to crop growth in many parts of the world. Nitrogen is essential to plant growth as it is a constituent of DNA, RNA, ATP, protein, chlorophyll, auxins, cytokinins, alkaloids and glucosinolates (Sprent, 1989; Grewal, 2010; Raven and Andrews, 2010; Hawkesford *et al.*, 2012; Andrews *et al.*, 2013; Udvardi and Poole, 2013). Phosphorus also is an essential element for plant growth as it is a constituent of DNA, RNA, ATP, phosphoproteins, phospholipids, and sugar phosphates (Bucher, 2007; Hawkesford *et al.*, 2012; Niu *et al.*, 2013). Water is required for photosynthesis, cell expansion, maintenance of cell turgor, short and long distance transport process and evaporative cooling (Cameron *et al.*, 2013; Farmer and Browse, 2013; Sauter, 2013).

Generally, in intensive agricultural systems, N and P limitations to crop growth are overcome by adding inorganic nutrient fertilisers and water limitations are countered by irrigation. Use of inorganic fertilisers and irrigation have been linked to environmental problems (Chapter 1). Also, in extensive systems, large inputs of N, P and water are unlikely to be commercially viable (McColl and Gibson, 1979; Scott, 2000; Monaghan *et al.*, 2005; Schon *et al.*, 2011; McDowell and Condron, 2012). Nitrogen fixing legumes can be utilized as the main input of N into agricultural systems. Here we focus on lucerne in New Zealand.

2.2 Lucerne in New Zealand

Lucerne also known as alfalfa is a perennial legume. It can fix substantial amounts of N in low N soils if other factors are not restricting growth. Also, its roots can grow to several metres depth and access water and nutrients that species with shorter roots cannot (Gregory, 2006; Teixeira *et al.*, 2009; Raza, 2010; Yang *et al.*, 2011). Linked to its ability to fix N, its leaves usually have high protein levels and are an excellent source of protein for animals (Moot *et al.*, 2008; Grewal, 2010; Moir and Moot, 2010). It also is usually high in Ca and other minerals.

Lucerne is a small seeded crop and takes several months to establish (White, 1967; Brooks *et al.*, 1982; Stout *et al.*, 1997; Guo *et al.*, 2010; Moot *et al.*, 2012; SpecialtySeeds, 2013). The study here focuses on the establishment phase.

Lucerne can be autumn or spring sown in New Zealand. It requires fairly high P (and K) levels for sustained growth. Recommended soil P levels for lucerne are 0.26-0.70 % in the top 15cm at a vegetative growth stage (PIONEER, 2013; SpecialtySeeds, 2013). Lucerne is intolerant of acid soils and related aluminium (Al) toxicity (Rechcigl *et al.*, 1988; Su and Evans, 1996; Moir and Moot, 2010). The recommended soil pH for successful establishment of lucerne is pH 6.0-6.5 (White, 1970).

Once established, lucerne can persist for 5 to 20 years depending on environmental conditions and variety and can be grazed several times a year (Moot *et al.*, 2008). The value of the nitrogen fixed by lucerne and other dryland legumes was estimated as $210 million per year for the South Island dryland alone (Brown and Green, 2003). Brown, Moot and McKenzie (2005a) ran an experiment over five growing seasons (1997-2002) and measured the yield of a lucerne crop on a Wakanui silt loam soil in dryland Canterbury. In the first full year of production, the stand produced 21t DM ha^{-1}. This shows that, if established correctly lucerne has the potential to survive and be productive under dryland conditions and to increase the potential productivity of dryland farms.

2.3 Lucerne and temperature

Generally, temperature is the main factor which drives lucerne yield and development and several authors have reported a linear relationship between the rate of development and temperature above a critical threshold (base temperature) (Moot *et al.*, 2000; Hay and Porter, 2006). Many reports have shown the importance of temperature on lucerne shoot and flowering development (Brown *et al.*, 2005a; Teixeira *et al.*, 2007). Leaf area at full expansion and hence light interception are greatly influenced by temperature, reaching maximum at about 20^{o}C and decreasing gradually at the lowered temperature (Field and Hunt, 1974; Robertson *et al.*, 2002). Stem diameter and stem weight are greater under cooler conditions. At 35^{o}C, there is an inhibition of floral initiation which can cause delay in flowering. Growth rates appear to be highest when daylight

temperatures are in the region of 20-25^{o}C (Pearson and Hunt, 1972). Water uptake by lucerne decreases with decreased temperature from 20^{o}C to 5^{o}C (Ehrler, 1963). Lucerne is a long day plant, and needs minimum photoperiod to initiate blossoming (Major *et al.*, 1991).

2.4 Lucerne and water

In crop plants, water use efficiency plays an important role when rainfall volume decreases. Lucerne roots can grow to several metres depth and access water and nutrients that species with shorter roots cannot (Figure 2.1).

In New Zealand, most lucerne is grown in the South Island (Avery *et al.*, 2008). In dry summers in Canterbury, lucerne consistently out yields other forage plants such as perennial ryegrass and white clover (Douglas, 1986; Brown *et al.*, 2006a). Moot (2008) stated that water use efficiency is the ratio of total dry matter accumulation to total water input to the system on an annual basis. Lucerne has a high water use efficiency in the range of 5-9 cm evapotranspiration per ha per ton of dry matter (Collino *et al.*, 2005) due to its ability to extract water from deeper layers in the soil (Evans, 1977).

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Figure 2.1 A comparison of the root systems of Left: perennial (*M. sativa*) and Right: annual (*M. bonarotiana*) species of *Medicago* (Weaver, 1926).

For example, lucerne was recorded to grow well on a deep silt loam soil in Christchurch with a high annual water use efficiency of 40 kg DM ha^{-1} mm^{-1} extracted 328mm of water from a depth of more than 2m (Moot *et al.*, 2008), meanwhile, perennial ryegrass only had a water use efficiency of 18 kg DM ha^{-1} mm^{-1} extracted 243mm of water from a depth of 1.5m (Figure 2.2).

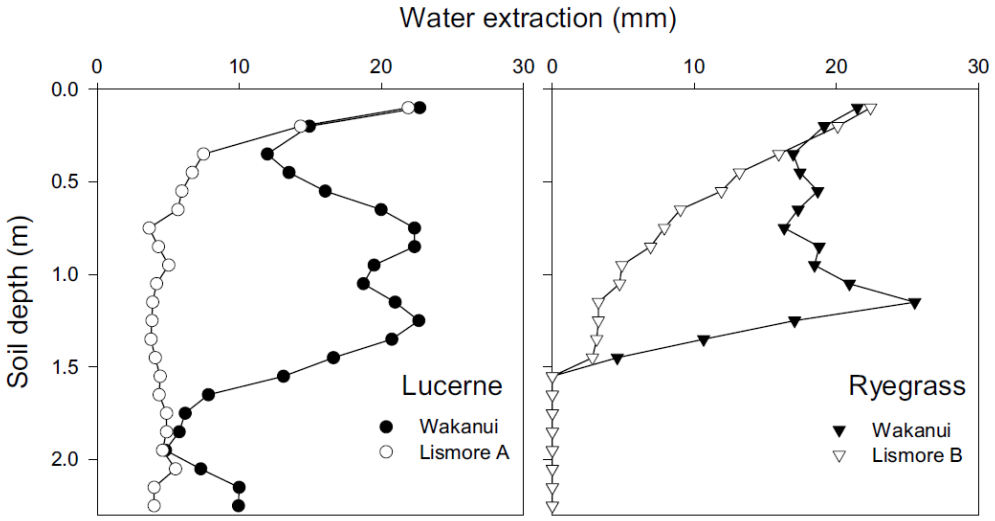

Figure 2.2 Water extraction (mm) from each 0.1 m soil layer from 0 – 2.3 m depth for lucerne (circles) and grass based pasture (triangles) on a deep Wakanui silt loam (solid symbols) or a Lismore (A) very stony loam and Lismore (B) stony loam (open symbols) (Moot *et al.*, 2008).

Lucerne is highly susceptible to water logging due to lack of oxygen reaching the deep tap root and reducing its function (Cocks, 2001).

2.5 Lucerne and N and P

Most plants can take up and utilize inorganic and organic forms of N from the soil (Andrews *et al.*, 2013). The main forms of N taken up and utilized by most crops are NO_3^- (nitrate) and NH_4^+ (ammonium) but amino acid, urea and proteins can be important in some situations (Andrews *et al.*, 2013). Nitrate and ammonium are primarily taken up by plant roots whilst organic N is generally taken up by mycorrhizas associated with roots, then transferred to the plants. Ammonium taken up by roots is primarily assimilated into amino acids in roots via the glutamine synthetase (GS)/glutamate synthase (GOGAT) pathway (Layzell, 1990; Andrews *et al.*, 2013). Nitrate taken up by roots must be reduced to ammonium before it can be assimilated into amino acids via the GS/GOGAT pathway

(Andrews *et al.*, 2013). This reduction occurs in two steps, the enzyme nitrate reductase (NR) reduces nitrate (NO_3^-) to nitrite (NO_2^-) then the enzyme nitrite reductase (NiR) reduces NO_2^- to NH_4^+ (Layzell, 1990; Andrews *et al.*, 2013). The root or shoot can be the main site of NO_3^- assimilation depending on plant species, cultivar and environmental conditions, especially soil NO_3^- level.

Lucerne can take up and utilize NO_3^- and NH_4^+ from the soil and it also forms associations with arbuscular mycorrhizal (Barea *et al.*, 1996; Vázquez *et al.*, 2002; Zhang *et al.*, 2011). Lucerne like many legumes can also carry out atmosphere N_2 fixation via symbiotic bacteria (general term rhizobia) in root nodules. The reaction is carried out by the rhizobial enzyme nitrogenase and is as follows (Layzell and Hunt, 1990; Andrews *et al.*, 2009a):

$$N_2 + 8H^+ + 8e^- + 16\ ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16\ Pi$$

Ammonium which is produced by nitrogenase in legume root nodules is assimilated into amino acids by the GS/GOGAT pathway in plant cells (Lea and Morot-Gaudry, 2001). The glutamate is transformed into different amino acids and used to construct N containing compounds as for glutamate produced in NH_4^+ and NO_3^- assimilation (Layzell and Hunt, 1990).

Lucerne nodules show indeterminate growth (Sprent, 2009). They have an apical meristem which results in a continuously growing nodule, these nodules are often cylindrical in shape with branches (Sprent, 2009). The most common rhizobia associated with lucerne is *Ensifer meliloti* and it is used in commercial inoculum, but other rhizobial strains can nodulate lucerne (See Chapter 3). *Ensifer meliloti* was originally named *Rhizobium meliloti* then *Sinorhizobium meliloti* before becoming now *Ensifer meliloti* (Jordan, 1984; de Lajudie *et al.*, 1994; Jarvis *et al.*, 1996; Young, 2003). Generally, N_2 fixation decreases with increased utilisation of soil N (Andrews *et al.*, 2009a).

Phosphorus is an essential element for plant/crop growth (see Chapter 1) but P levels in New Zealand soils severely limit crop (including lucerne) growth (Smith and Cornforth, 1982; Wheeler and Edmeades, 1995; McDowell and Condron, 2004; Maxwell *et al.*,

2012). In order to sustain high dry matter production and N_2 fixation, lucerne requires substantial P availability as well as K, Ca and micronutrients, in particular, B, and Mo which is a component of the nitrogenase enzyme (Scott and Archie, 1978; Valenciano *et al.*, 2011).

Phosphorus and water uptake have been shown to be stimulated by vesicular arbuscular mycorrhiza (Barea *et al.*, 1980; Barea and Azcon-Aguilar, 1983; Vázquez *et al.*, 2002; Kahiluoto *et al.*, 2012; Jannoura *et al.*, 2013; Verbruggen *et al.*, 2013; Zhang *et al.*, 2013). The tri-symbiosis between lucerne, rhizobia and mycorrhiza has been found to increase quality and lucerne yield (Vázquez *et al.*, 2002).

2.6 Lucerne and rhizobial inoculant

Ensifer meliloti is the main rhizobial species associated with nodulation and nitrogen fixation in lucerne and is the rhizobia used in commercial inoculum for lucerne (Frame, 2005). *Ensifer meliloti* does not occur naturally in New Zealand soils and it is recommended that rhizobial inoculant should be applied to lucerne in New Zealand soils (Greenwood, 1964; Hastings *et al.*, 1966; Deaker *et al.*, 2004). As long ago as 1929, Reid (1929) showed that effective lucerne nodulation could fix about 450kg N per ha per year from the atmosphere.

Level of nodulation and subsequent N_2 fixation depend on rhizobial strains, lucerne cultivar and environmental conditions (Burton, 1972; 1981). According to Graham (1992), *E. meliloti* occurrence is reduced below pH 6 and is affected by the presence of mineral N. In most cases in New Zealand, commercial lucerne inoculation is recommended for maximum crop productivity. However recent results for white clover (*Trifolium repens*) indicate that if sufficient effective rhizobia are already present in the soil, as a result of a previous inoculation, further inoculation may not be necessary (Lowther and Kerr, 2011).

There is substantial evidence that successful inoculation requires large numbers of viable rhizobia per seed. According to the Australian Legume Inoculants Research Unit (ALIRU), a minimum of 1000 effective rhizobia are required per seed for effective nodulation (Thies *et al.*, 2001). Parle *et al.* (1973) argued that high viable rhizobial counts in

inoculants do not always lead to satisfactory counts on inoculated seed. This was consistent with Blair (1960; 1971) who stated that survival of viable rhizobia on seed was of far greater importance than their quantity. Lowther and Patrick (1995) concluded that rhizobia strains with high survival rates normally showed high effective nodulation after sowing.

Inoculants are produced in a carrier material which may be added directly to the seed or placed in the furrow prior to sowing. Peat is the most commonly used carrier for rhizobial inoculants mainly because of its high moisture holding capacity and dual ability to foster multiplication of rhizobia and protect it once applied to the seed coat but other carriers are possible (Herridge, 2002). Peat-based inoculants are thought to give a considerable measure of protection to the rhizobia on the seed surface (Parle *et al.*, 1973).

Sterile and non-sterile peats are common carriers for rhizobia although sterile peats are generally preferred by farmers because they contain up to 100 fold more rhizobia than nonsterile types (Thompson, 1983). They are, however, costly, easily contaminated, require strict asceptic maintenance and suffer consistent unsatisfactory nodulation of new stands particularly when dry weather prevails (Wynn-Williams, 1976). Peat inoculum reduces rhizospheric pH due to the acidic nature of most peat deposits (Roughley, 1970). Pre-mixing peat with seed prior to sowing is laborious and time consuming especially when establishing large fields (Werner *et al.*, 2005).

In some cases, competition for nodulation between the rhizobial strain in the inoculum and less effective indigenous soil rhizobial strains can reduce the efficiency of the inoculum (Bromfield, 1984; 1986; Triplett and Sadowsky, 1992; Thies *et al.*, 2001). This could be relevant to the use of inoculum with lucerne in New Zealand. Specifically, Khumalo (2011) and Wigley (2012) compared application of commercial inoculum on lucerne via different carriers namely peat, lime coating and ALOSCA granules in dryland Canterbury. ALOSCA technology is based on a bentonite clay granule that contains high numbers of viable rhizobial. Regardless of carrier, genotype characterization of bacteria isolated from root nodules of inoculated plants contained a substantial proportion of *Ensifer meliloti* as expected but also a substantial proportion of *Rhizobium* sp. It was

shown that these *Rhizobium* sp. produced nodules but it is not known if these nodules fixed atmosphere nitrogen. In Experiment 1 here (Chapter 3), we compare growth of lucerne supplied four of these *Rhizobium* strains to lucerne supplied two strains of *Ensifer meliloti*.

2.7 Lucerne and mycorrhizal inoculant

About 80% of terrestrial plant species including most crop species and lucerne are capable of forming a symbiotic relationship with vesicular arbuscular mycorrhizas (VAM) (Liu *et al.*, 2000; Smith *et al.*, 2001; Bowman *et al.*, 2002; Liu *et al.*, 2004; Asghari *et al.*, 2005; Smith *et al.*, 2011). There is strong evidence to show that mycorrhizal colonization can be of great benefit to plants but that effects of colonization vary markedly depending on environmental conditions and species of both host plant and fungus (Smith and Read, 2008). Similarly, the effects of mycorrhizal inoculant on crop growth are variable (Hodge and Andrews, 2004; Andrews *et al.*, 2010). The benefit to host plants are assumed to be because mycorrhizal colonization may improve nutrient (especially P) uptake, water relations, resistance to soil pathogens, and resistance more generally to adverse soil conditions. Furthermore, since mycorrhizas may infect many different host species, the mycelial network they develop below ground may connect plants and facilitate transfers of carbon and nutrients among them (Raven, 2010).

In relation to P uptake, it has been shown that the external hyphae of mycorrhizas can absorb P from outside the root depletion zone and transport it to the host plant (Asghari *et al.*, 2005; Ridgway *et al.*, 2006, 2008). The rate of P nutrition absorption by roots or mycorrhizas is known to depend on the rate of nutrient supply to the rhizosphere, this being influenced by the mobility of the phosphate ions and its concentration in the soil solution (Chapin, 1980). Therefore, VAM enhance P uptake by increased number of sites for absorption achieved by the external mycelium. The hyphae growing through soil pore spaces are able to elicit phosphate absorption beyond the depletion zone up to 8 cm from the root (Rhodes and Gerdemann, 1975). Thus, mycorrhizal roots explore a much greater volume of soil to take up phosphate.

Vesicular arbuscular mycorrhizas are present in virtually all soils, but mycorrhizal population levels may differ greatly under various ecological conditions. Indigenous mycorrhizal populations can be diminished by agricultural practices such as heavy P fertilization and pesticide treatments. The coexistence of a bacterium and a fungus as root endophytes of legumes was first reported by Asai (1944). The role of mycorrhizas in the growth, nodulation, and N fixation of legumes has been a subject of increasing interest (Brockwell and Hely, 1966; Powell and Daniel, 1978; Barea *et al.*, 1980; Azcon-Aguilar and Barea, 1981; Azcón *et al.*, 1991; Asghari *et al.*, 2005; Smith *et al.*, 2011; Wang *et al.*, 2012). In the context of these interactions, it should be stated that the formation of VAM entry points and nodules on a legume root occurs simultaneously, usually within a few days after seed inoculation. And it appears that the two endophytes do not compete for infection sites (Mosse, 1973).

Mycorrhiza can stimulate nodulation/nitrogen fixation. The double symbiosis in legumes has been found to fix more N than those nodulated but nonmycorrhizal (Kucey and Paul, 1982). The existence of a direct P supply to the nodules via the mycorrhizal hyphae is a condition for effective symbiotic N fixation (Crush, 1976). This is not only because of the role of the host as a partner in the association as concerns the expression of the N fixation, but also because the nodules are actually part of the plant. If the plant is well nourished, the nodules will also receive suitable P for their efficient functioning.

Mosse (1978) found that plants did not nodulate unless their P concentrations were at least 0.15%; mycorrhizal infection helped the plants to reach this required level, and nodulation then occurred. The mycorrhizal effects on nodulation take place through host nutrition and these occur at the same time as the growth responses.

In this thesis, the effect of mycorrhizal inoculum on growth of lucerne plus or minus rhizobial inoculum and under different levels of P is determined.

2.8 Summary of literature on lucerne

- Lucerne can be sown in late spring or autumn in New Zealand provided soil moisture and temperature conditions are favourable
- Lucerne is capable of high dry matter yields
- Lucerne is particularly suited to dryland conditions due to its deep tap root
- Lucerne forms a symbiotic relationship with *Ensifer meliloti* that allows it to fix high amounts of atmospheric N although this is dependent on soil N, P and water availability. It is recommended that *Ensifer meliloti* inoculum is applied to lucerne crops in New Zealand. Recent work has shown that rhizobia other than *Ensifer meliloti* can nodulate lucerne in New Zealand soils.
- Outside of New Zealand, mycorrhizal inoculum has been shown to increase growth and yield of lucerne under some conditions. In some cases, increased growth and yield has been linked with increased P uptake.
- Lucerne is intolerant of acidic/low pH (<5.8) soils – optimum growth occurs at pH of 6.0 – 6.3
- Lucerne has low resistance to pest/insects but cultivars with greater pest and disease resistance have been produced.

Chapter 3. EFFECTS OF DIFFERENT RHIZOBIAL STRAINS ON GROWTH OF LUCERNE UNDER DIFFERENT N AND P LEVELS

3.1 Introduction

Legumes are of economical importance in many agricultural systems due to their ability to fix atmospheric N_2 (Gault *et al.*, 1995; Peoples *et al.*, 1996). For legumes growing in a low soil N environment, the ability to fix atmospheric N_2 is advantageous but there are many reports that the proportion of total plant N obtained from N_2 fixation decreases with increased soil N availability under managed/controlled conditions (Andrews *et al.*, 2011b). Cost benefit analysis on the basis of biochemical principles indicate that utilisation of NH_4^+, NO_3^- and organic N from soil sources is energetically more efficient than N_2 fixation in high soil N environments (Andrews *et al.*, 2011a). The ability of legumes to fix atmospheric N_2 and utilise soil N is dependent on species, as is relative growth potential when reliant on N_2 fixation or soil N (Andrews *et al.*, 2004; 2009a). One of the limitations for legume N_2 fixation is that there is a lag phase from germination to the production of fully functional nodules. The addition of 'starter' N (approximately 20 kg/ha) may improve early legume growth and enhance the nodulation process in low soil N (Andrews *et al.*, 2009b). On the other hand, many studies reported that increasing N applied to lucerne up to 100 kg/ha caused a reduction in number of functional nodules as well as dry matter yield (Vance *et al.*, 1979; Eardly *et al.*, 1985).

Lucerne is a temperate perennial forage legume which is an important crop plant in New Zealand with considerable potential of expansion in some farming systems (Popay *et al.*, 2010). Lucerne, which is native to the Mediterranean and western Asia, has a deep stout taproot which allows the plant to obtain more macro-nutrients such as soil mineral N, and soil P and also micro-nutrients, and water. Early studies indicated that the rhizobial population in New Zealand native soil were unable to nodulate lucerne and thus, it is common practice these days to use rhizobial inoculants imported from Australia to grow lucerne in New Zealand (Khumalo, 2011; Wigley *et al.*, 2012).

Ensifer meliloti is the main rhizobial species associated with nodulation and nitrogen fixation in lucerne and is the rhizobia used in commercial inoculum for lucerne (Frame, 2005; Lakzian *et al.*, 2008; Ardakani *et al.*, 2009b; Bromfield *et al.*, 2010; Redondo *et al.*, 2012). *Ensifer meliloti* does not occur naturally in New Zealand soils and it is recommended that rhizobial inoculant should be applied to lucerne in New Zealand (Greenwood, 1964; Hastings *et al.*, 1966; Deaker *et al.*, 2004). Reid (1929) showed that effective lucerne nodulation could fix about 450kg N per ha per year from the atmosphere.

Level of nodulation and subsequent N_2 fixation depend on rhizobial strains, lucerne cultivar and environmental conditions (Burton, 1972; 1981). In some cases, competition between the rhizobial strain in the inoculum and less effective indigenous soil rhizobial strains for nodulation can reduce the efficiency of the inoculum (Bromfield, 1984; 1986; Triplett and Sadowsky, 1992; Thies *et al.*, 2001). This could be relevant to the use of inoculum with lucerne in New Zealand. Specifically, Khumalo (2011) and Wigley (2012) compared application of commercial inoculum on lucerne via different carriers, namely peat, lime coating or ALOSCA granules, in dryland Canterbury. Regardless of carrier, genotypic characterization of bacteria isolated from root nodules of inoculated plants indicated that a substantial proportion of nodules contained *Ensifer meliloti* as expected but also a substantial proportion of nodules contained *Rhizobium* sp. It was shown that these *Rhizobium* sp. produced nodules but it is not known if these nodules fixed atmosphere nitrogen.

The main objectives of this chapter were firstly to test under different N and P availability, the effectiveness of *Ensifer meliloti* and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand (Khumalo, 2011; Wigley *et al.*, 2012) on growth of lucerne. On finding that the strains of *Rhizobium* sp. gave poor growth of lucerne in comparison with *Ensifer meliloti* or uninoculated plants, work on *Rhizobium* sp. was not continued after the first experiment. Secondly, growth of lucerne relying primarily on N_2 fixation was matched against its growth potential with optimum soil N.

3.2 Materials and methods

3.2.1 Experiment 1: Effect of *Ensifer meliloti* and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand on growth of lucerne

Experiment 1 had 8 treatments, two strains of *Ensifer meliloti* and its mixture, four strains of *Rhizobium* sp. and a control with no rhizobia in one level of nitrogen, 25kg N/ha and one level of phosphorus, 4.5kg P/ha with 6 replicates (6 pots) (Figure 3.1). The experiment started on 21st September 2012 and was harvested after 9 weeks on 30th November 2012 for shoot fresh weight, and shoot and root dry weight.

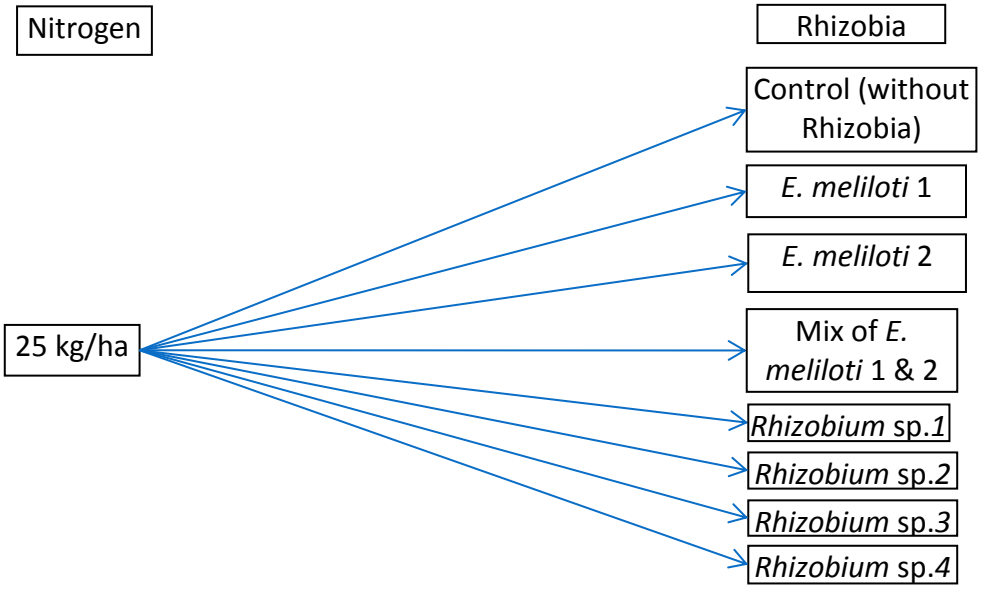

Figure 3.1 Treatments in experiment 1

Experiment 1 was conducted in a temperature controlled glasshouse at Lincoln University, New Zealand in a randomised complete block design. Lucerne was grown in 4 litre pots with 18cm diameter containing a basal potting mix media, with N and P added as required. One tonne of basal potting mix media (no N or P) contained 800L bark, 200L pumice, 300g Osmocote (5-6 months) 0N – 0P – 37K, 1000g Horticultural lime, 300g Macromax, 1000g Hydrafts. Soluble N (Calcium Ammonium Nitrate, 27%N) was added at the rate of 25kg/ha to the pots directly and P (Super phosphate, 9%P) ground fine powder was mixed well with potting mix prior to sowing at 4.5kg/ha.

Pots were filled up with media prior to sowing lucerne seed 'Stamina 5' (PGG Wrightson) at a depth of 1 cm with the rate 8 kg/ha = 10 plants/pot as in the recommended field rate (Moot *et al.*, 2000; 2012). Pots were inoculated with four rhizobial strains shown to produce nodules by previous authors (Khumalo, 2011; Wigley *et al.*, 2012) and two *Ensifer meliloti* strains and a mixture of the two shown to produce N_2 fixing nodules (Liu, 2013).

The rhizobia were removed from stored YMA plates and used to inoculate 1 mL of yeast mannitol broth (YMB; 0.1% (w/v) yeast extract, 1% (w/v) mannitol, 0.0005 mM dipotassium phosphate, 0.0002 mM magnesium sulphate, 0.0001 mM sodium chloride, autoclaved in a sterile 2 mL tube. This liquid suspension was incubated in a shaking incubator at 28^{o}C for 24-48 h at 220 rpm (LABNET 211 DS, Labnet International, USA). Lucerne seeds were inoculated with soluble mixture (1 ml containing 10^8 cells) of the appropriate rhizobium culture directly after sowing into the pots.

Plants were grown in a glasshouse (13-16h daylight) with temperature in the range of 10-25°C during Spring and Summer 2012. Pots were irrigated with the amount of 100-250ml tap water every two days when the potting mix water content was lower than 60% relative water content (3 pots randomly selected were weighed to calculate the relative water content).

Seedlings were thinned to ten plants per pot after emergence and re-inoculated with soluble rhizobia after 10 days to insure that all pots were uniformly inoculated. The plants were harvested by cutting shoots 2 cm above ground level at the crown according to 50% budding or 10% flowering appearance. Shoot and root dry weights were obtained after drying at 70^{o}C for 72 hours.

3.2.2 Experiment 2: Effect of *Ensifer meliloti* inoculum on growth of lucerne under different N and P levels - one harvest

Experiment 2 had 8 treatments with two levels of phosphorus, two treatments of rhizobia and two levels of nitrogen with 6 replicates (6 pots) (Figure 3.2). It started on 15th August 2012 and was harvested on 29th October 2012 after 9 weeks for shoots and roots dry weight and 'quality' measurements.

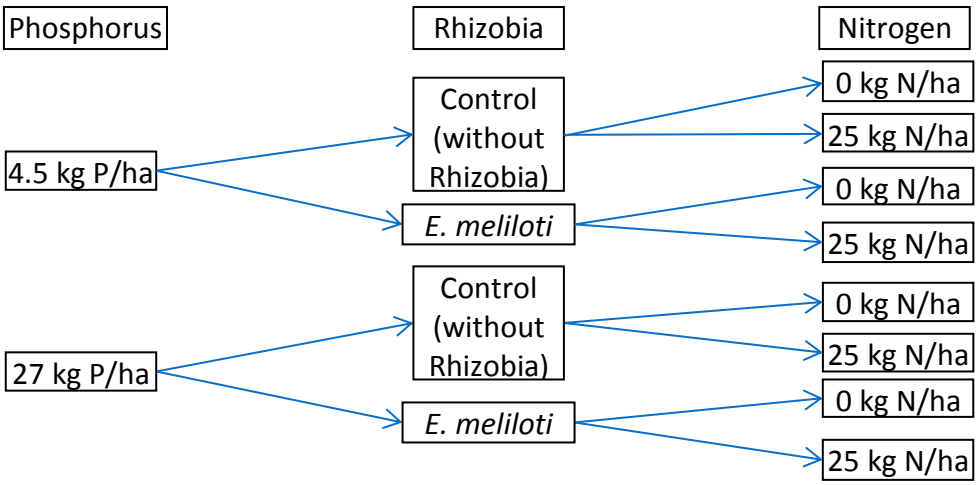

Figure 3.2 Treatments in experiment 2

This experiment and all glasshouse experiment hereafter used the same basal potting mix media, pots, seeds, and glasshouse as in experiment 1. In experiment 2, there were two levels of Phosphorus (4.5kg/ha and 27kg/ha), two treatments of rhizobia (control and *E. meliloti*), and two levels of Nitrogen (0kg/ha and 25kg/ha).

After harvesting, shoot fresh weight and dry weight, and root dry weight were measured as in experiment 1. Sub-samples of dried shoot material were analyzed for the quality measurements: Crude Protein (CP), Dry Matter Digestibility (DMD) and Metabolisable Energy (ME) by NiR-spectrometer 5000M machine.

An overview of experiment 2 is shown in Plate 3.1.

Plate 3.1 Early stage of glasshouse experiment 2

3.2.3 Experiment 3: Effect of *Ensifer meliloti* inoculum on growth of lucerne under different N and P levels - two harvests

Experiment 3, as for experiment 2 had 8 treatments with two levels of phosphorus, two levels of rhizobia and two levels of nitrogen with 6 replicates (6 pots) but there were two harvests (Figure 3.3). The trial started on 16th November 2012 with a shoot cutting on 21st January 2013 and another on 14th March 2013. Shoot fresh weight and dry weight were measured at the first harvest. Shoot fresh weight and dry weight and root dry weight were measured at the second harvest.

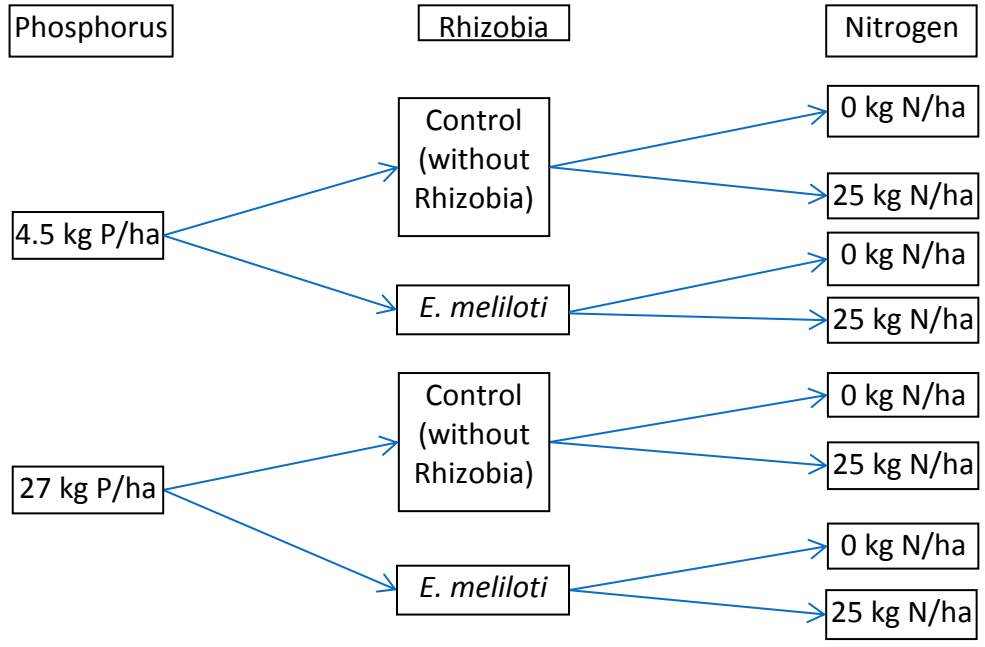

Figure 3.3 Treatments in experiment 3

3.2.4 Experiment 4: Effect of different N levels on *E. meliloti* inoculated lucerne

Experiment 4 had seven levels of nitrogen (0kg/ha, 25kg/ha, 50kg/ha, 75kg/ha, 100kg/ha, 150kg/ha, 200kg/ha) with 4 replicates (4 pots) (Figure 3.4). All pots were inoculated with *Ensifer meliloti.* The experiment started on 16th November 2012 with a shoot cutting on 21st January 2013 and another on 14th March 2013. Experiment 4 used the same basal potting mix as experiment 1 with added 4.5kg P/ha. Shoot fresh weight and dry weight and root dry weight were determined at harvest.

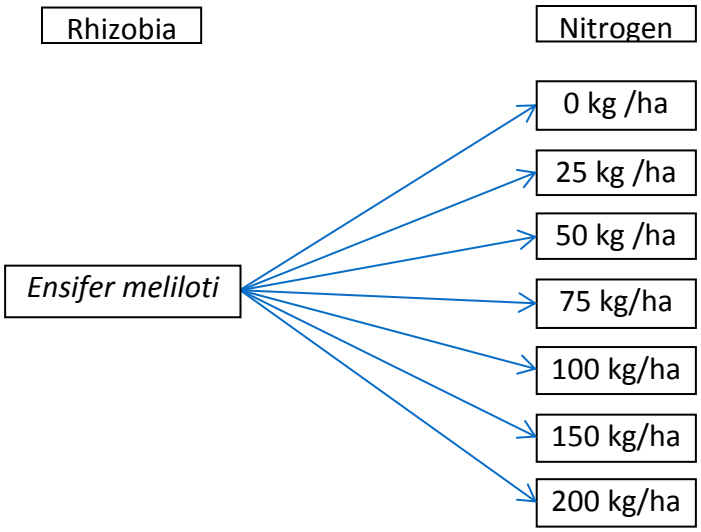

Figure 3.4 Treatments in experiment 4

3.2.5 Statistical analyses

Statistical analyses were performed using Genstat version 14.1 (VSN International Ltd, UK, 2011). All variables were analysed via balanced multi-factor analysis of variance. All effects discussed had a probability $P<0.05$.

3.3 Results

3.3.1 Experiment 1: Effect of *Ensifer meliloti* and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand on growth of lucerne

The two strains and mix of *Ensifer meliloti* increased total plant dry matter of lucerne versus the control treatment. However, all the *Rhizobium* sp. isolated from New Zealand soils decreased total plant dry matter (Figure 3.5; Table 3.1). There was an increase of 14% in shoot dry weight and 33% in total plant dry weight for the *E. meliloti* treatment over the control.

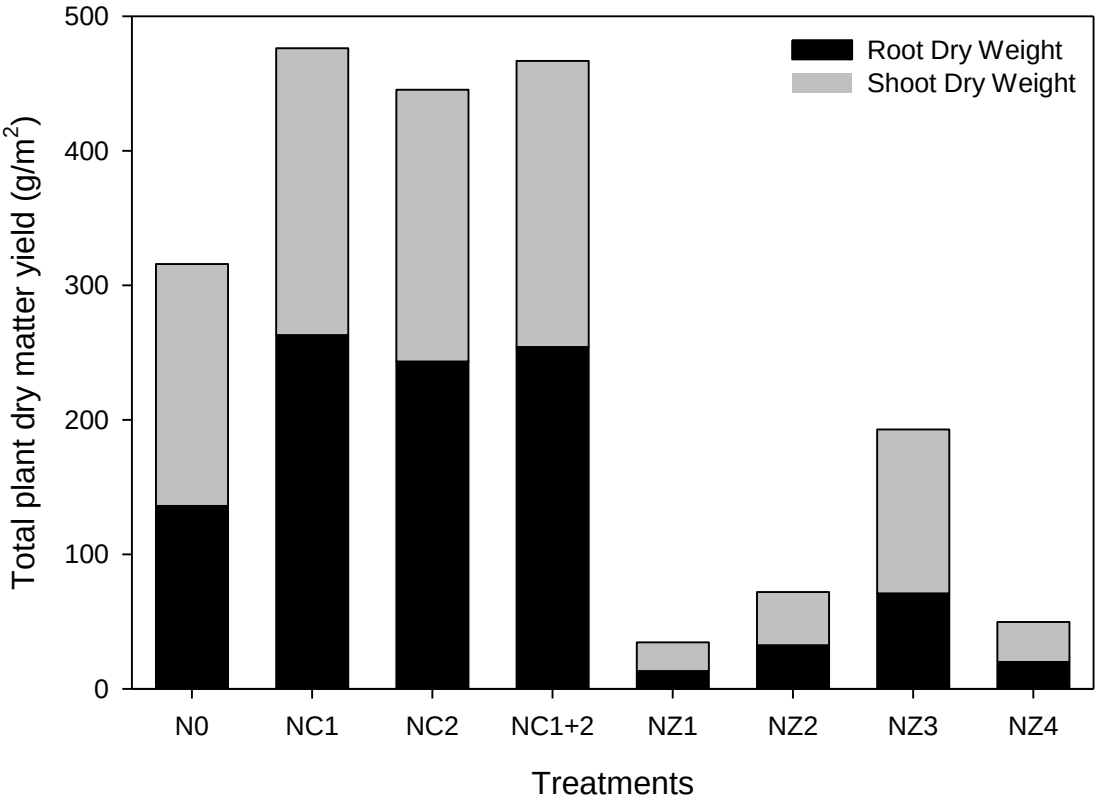

Figure 3.5 DM yield of shoot and root of lucerne with different strains of rhizobia (g/m^2)

N0 = control; NC = *E. meliloti* (commercial inoculum); NZ = *Rhizobium* sp. (Strains isolated from lucerne in New Zealand soils).

Shoot to root dry weight ratio was similar to the control with the strains of *Rhizobium* sp. but lower than control with the *E. meliloti* strains (Table 3.1). Shoot water content ranged

from 77.53% to 81.23% with no consistent difference between *E. meliloti* and *Rhizobium* sp. treatments.

Table 3.1 Total plant dry matter (TPDW) and shoot to root dry weight ratio (S:R) of lucerne with different strains of rhizobia in experiment 1.

Inoculum	TPDW	S:R
Uninoculated, N0	315.7[b]	1.48[ab]
E. meliloti 1&2, NC	476.2[a]	0.81[c]
E. meliloti 1, NC1	445.4[a]	0.83[c]
E. meliloti 2, NC2	466.8[a]	0.84[c]
Rhizobium sp.1, NZ1	34.4[d]	1.60[a]
Rhizobium sp.2, NZ2	72.0[d]	1.23[b]
Rhizobium sp.3, NZ3	192.8[c]	1.77[a]
Rhizobium sp.4, NZ4	49.6[d]	1.49[ab]
Significance of F	***	***
Grand Mean	256.6	1.26

*** Significance of F at the level of probability P<0.001.
Means with the same letter in column are not significantly different (P<0.05).

Plate 3.2 shows roots of plants from selected treatments. Note the extensive nodulation with *E. meliloti* and small roots with *Rhizobium sp*.

Plate 3.2 Lucerne roots after cleaning with tap water (N0, NC, and NZ are Uninoculated, *E. meliloti* and *Rhizobium* sp., respectively)

In a small repeat experiment, a mixture of the four *Rhizobium* sp. strains also gave lower growth than the control (281.2 ± 1.67 g/m^2 against 341.7 ± 1.67 g/m^2).

3.3.2 Experiment 2: Effect of *Ensifer meliloti* inoculum on growth of lucerne under different N and P levels - one harvest

In experiment 2, *E. meliloti* and additional N and P all had significant positive effects on total plant dry weight and shoot dry weight (Figure 3.6; Table 3.2).

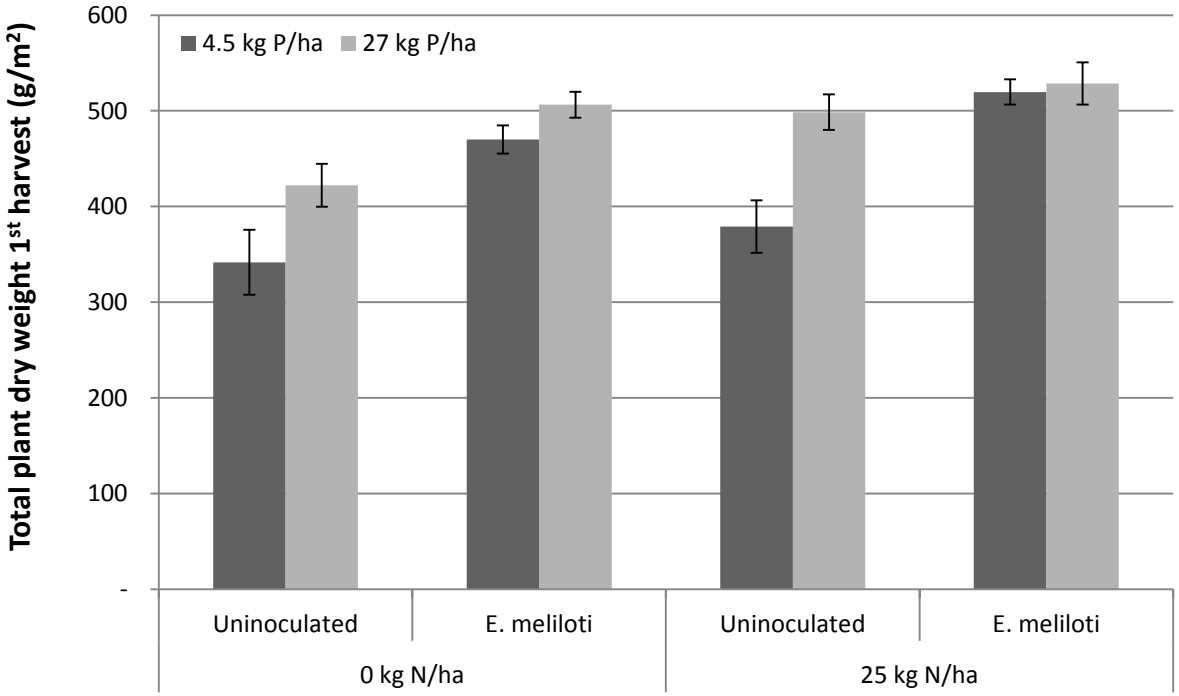

Figure 3.6 Effect of *E. meliloti* inoculum and additional N and P on total plant dry weight of lucerne in experiment 2. The vertical bar indicates standard error of mean.

Total plant dry weight was 52.1% greater with 25kg N/ha, *E.meliloti* inoculum and 27kg P/ha than with the uninoculated control with 0N and 4.5kg P/ha.

At low P but not high P, S:R was lower for plants inoculated with *E. meliloti* than uninoculated plants (Table 3.2).

Table 3.2 Total plant production (g/m^2), and shoot:root ratio of lucerne with inoculum of *E. meliloti, Rhizobium* sp. and two levels of P and two levels of N in experiment 2.

		Total plant DM		S:R	
Treatments	N	P1	P2	P1	P2
Uninoculated	-[1]	341.7^{e}	422.2^{cd}	1.10^{a}	0.83^{bc}
	+	379.0^{d}	498.6^{a}	1.01^{ab}	0.73^{c}
E. meliloti	-	469.9^{bc}	506.4^{a}	0.75^{c}	0.73^{c}
	+	519.7^{a}	528.7^{a}	0.75^{c}	0.76^{c}
Grand Mean		419.8		0.88	

[1] '+' and '-' represent with and without 25 kg N/ha
Means with the same letter in paired columns are not significantly different (P<0.05).

There was little difference in shoot water percentage across treatments, values ranged from 76.49% to 79.87%. Crude protein, dry matter digestibility and metabolisable energy were not affected by treatment (Table 3.3).

Table 3.3 Crude protein (CP), dry matter digestibility (DMD), and metabolisable energy (ME) with inoculum of *E. meliloti,* two levels of P and two levels of N

		CP (%)		DMD		ME	
Treatments	N	P1	P2	P1	P2	P1	P2
Uninoculated	-[1]	24.51^{ab}	23.74^{ab}	70.36^{a}	69.47^{a}	10.84^{a}	10.73^{a}
	+	25.47^{a}	24.06^{ab}	70.31^{a}	71.22^{a}	10.80^{a}	10.97^{a}
E. meliloti	-	24.27^{ab}	24.61^{ab}	69.14^{a}	70.59^{a}	10.71^{a}	10.88^{a}
	+	24.65^{ab}	24.16^{ab}	71.22^{a}	70.69^{a}	11.00^{a}	10.91^{a}
Grand Mean		24.19		70.30		10.83	

[1] '+' and '-' represent with and without 25 kg N/ha
Means with the same letter in paired columns are not significantly different (P<0.05).

Plants from selected treatments are shown in Plate 3.3.

Plate 3.3 Effects of *E. meliloti* inoculum, N and P on growth of lucerne in Experiment 2. P1 (P applied at 4.5kg/ha); P2 (P applied at 27kg/ha); R0 (Uninoculated); RC (Inoculated with *E. meliloti*); N0 (N applied at 0kg/ha); N2 (N applied at 25kg/ha).

3.3.3 Experiment 3: Effect of *Ensifer meliloti* inoculum on growth of lucerne under different N and P levels - two harvests

In the first harvest of experiment 3, as for experiment 2, *E. meliloti* inoculum and addition of N and P all had positive effects on shoot dry weight of lucerne (Figure 3.7). Shoot dry weight was 19.68% greater with 25kg N/ha, *E. meliloti* and 27kg P/ha than with the uninoculated control with 0N and 4.5kg P/ha. Shoot water content ranged from 74.99 – 76.47% and was unaffected by treatment.

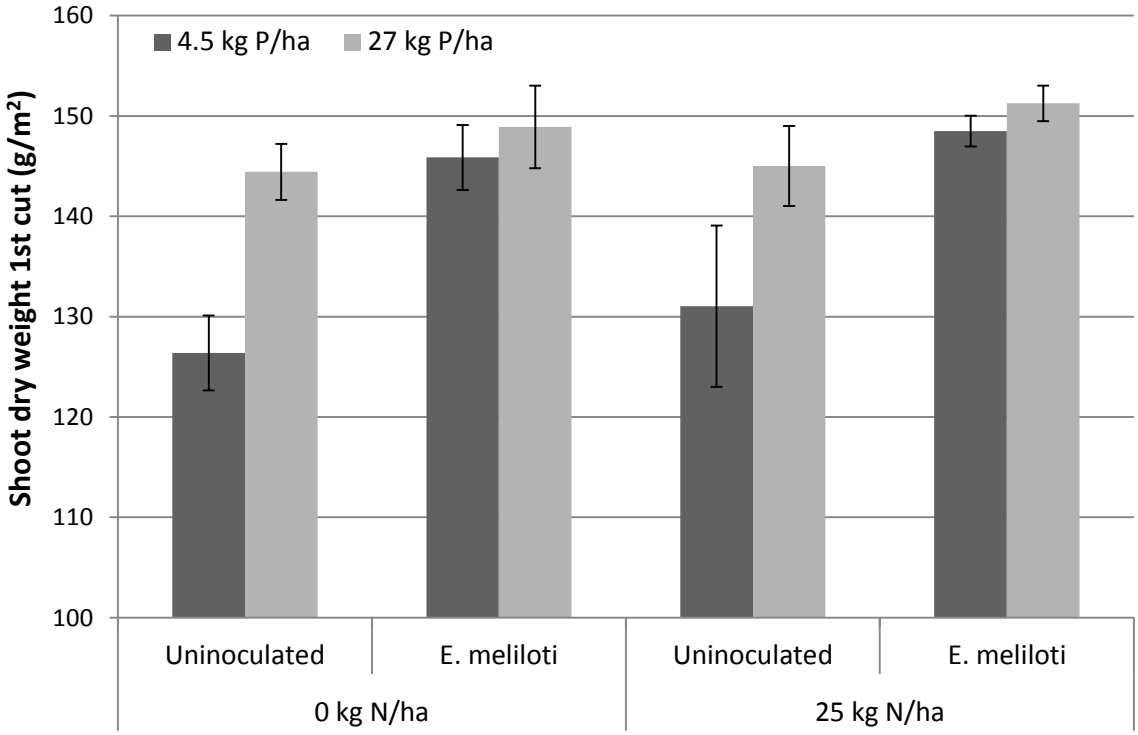

Figure 3.7 Effect of *E. meliloti* inoculum and additional N and P on shoot dry weight of lucerne at first harvest in experiment 3. The vertical bar indicates standard error of mean.

As for shoot dry weight in the first harvest of experiment 3, shoot dry weight and total plant dry weight at the second harvest increased with additional N, *E. meliloti* inoculum and additional P (Figure 3.8).

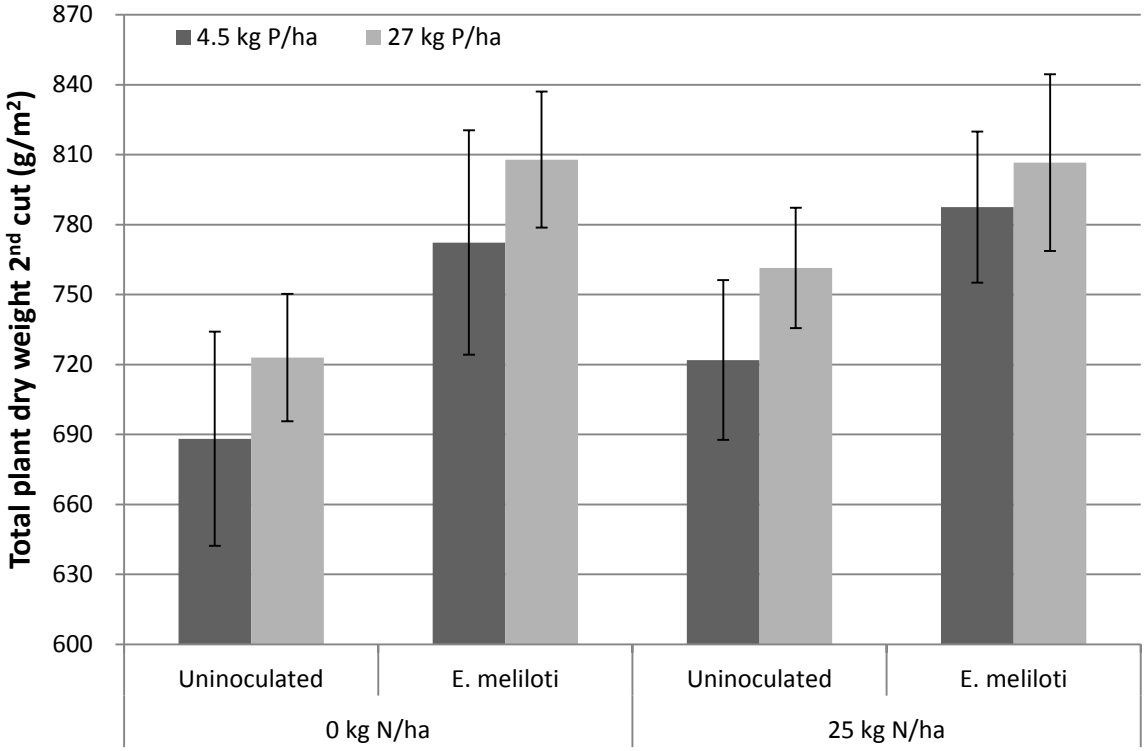

Figure 3.8 Effect of *E. meliloti* inoculum and additional N and P on total plant dry weight of lucerne at second harvest in experiment 3. The vertical bar indicates standard error of mean.

Total plant dry weight was 17.22% greater with 25kg N/ha, *E. meliloti* and 27kg P/ha than with the uninoculated control with 0N and 4.5kg P/ha. Shoot water content ranged from 76.86 – 78.97% and was unaffected by treatment.

At low P and high P, S:R was generally lower for plants inoculated with *E. meliloti* than for uninoculated plants (Table 3.4).

Table 3.4 Total plant production in 2nd cut (TPDW) (g/m^2), and shoot:root ratio of lucerne with inoculum of *E. meliloti* and two levels of P and two levels of N in experiment 3.

		TPDW2		S:R	
Treatments	N	P1	P2	P1	P2
Uninoculated	-[1]	688.1^{c}	723.0^{bc}	0.99^{a}	0.90^{ab}
	+	721.9^{bc}	761.4^{ab}	0.89^{ab}	0.88^{b}
E. meliloti	-	772.3^{ab}	807.9^{a}	0.88^{b}	0.82^{b}
	+	787.5^{a}	806.6^{a}	0.84^{b}	0.85^{b}
Grand Mean		758.6		0.88	

[1] '+' and '-' represent with and without 25 kg N/ha
Means with the same letter in paired columns are not significantly different (P<0.05).

3.3.4 Experiment 4: Effect of different N levels on *E. meliloti* inoculated lucerne

In the first harvest of experiment 4, shoot dry weight gradually increased from a mean of 130.5 to 143.7 g/m^2 with increasing added levels of N from 0 to 200 Kg/ha (Figure 3.9). This was a 10% increase in shoot dry weight.

Shoot water content ranged from 75.28% to 76.03% with no consistent difference between levels of N added from 0kg/ha to 200kg/ha.

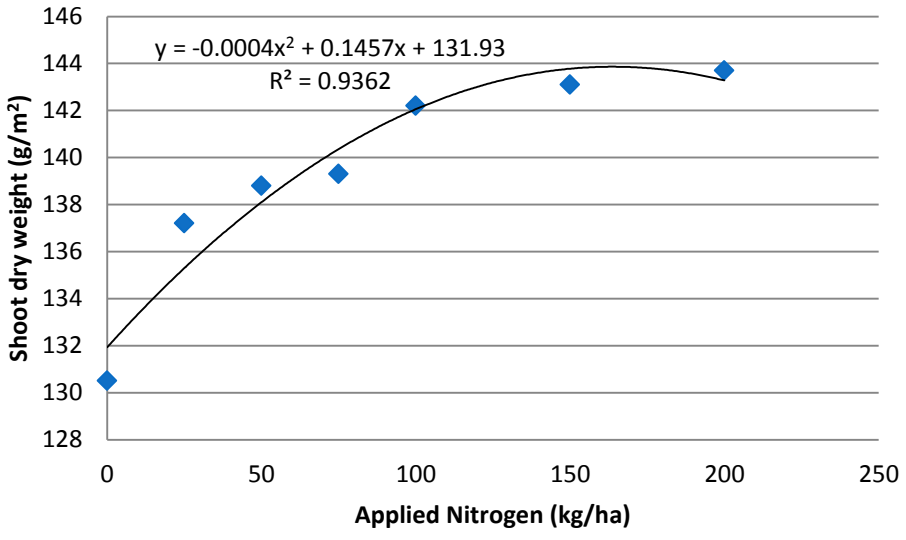

Figure 3.9 Effect of different rates of nitrogen on shoot dry weight of lucerne in experiment 4 – first harvest

For the second harvest, total plant dry weight of lucerne increased from 671.8 ± 10.87 g/m^2 at 0kg N/ha to 752.5 ± 10.87 g/m^2 at 50 kg N/ha then decreased with increased applied N thereafter (Figure 3.10). This was a 12% increase in total plant dry weight with increased applied N from 0 to 50 kg/ha.

Shoot water content ranged from 78.68% to 80.32% with no consistent difference between levels of N added from 0kg/ha to 200kg/ha.

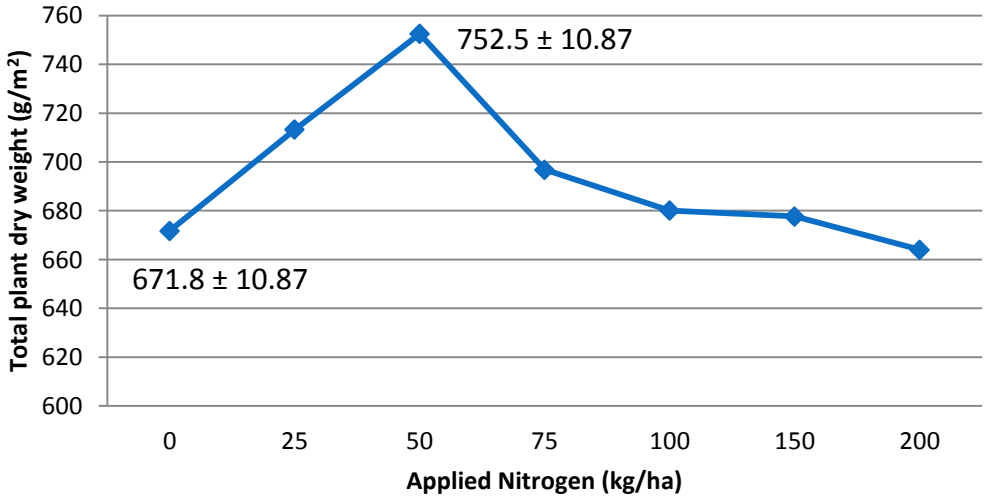

Figure 3.10 Effect of different rates of nitrogen on total plant dry weight of lucerne in experiment 4 – second harvest

The shoot to root dry weight ratio was lowest with treatment of 50kg N/ha and highest with treatment of 0kg N/ha (Table 3.5).

Table 3.5 Total plant production (g/m^2), and shoot:root ratio of lucerne with different levels of N, added *E. meliloti* in experiment 4.

Level of N (kg/ha)	TPDW	S:R
0	671.8^{bc}	0.90^{a}
25	713.3^{ab}	0.82^{ab}
50	752.5^{a}	0.78^{b}
75	696.8^{bc}	0.83^{ab}
100	680.1^{bc}	0.82^{ab}
150	677.7^{bc}	0.81^{ab}
200	664.0^{c}	0.80^{ab}
Significance of F	***	NS
Grand Mean	693.7	0.82
***, NS Significance of F at the level of probability P<0.001 and P>0.05, respectively. Means with the same letter in column are not significantly different (P<0.05).		

3.4 Discussion

The objectives of the experiments described in this chapter were to 1) test under different N and P availability, the effectiveness of *Ensifer meliloti* and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand on growth of lucerne and 2) determine how growth of lucerne relying primarily on N_2 fixation matched its potential growth with optimum soil N.

In experiment 1, the two strains and mix of *Ensifer meliloti* increased total plant dry matter of lucerne versus the control treatment. However, all the *Rhizobium* sp. isolated from New Zealand soils decreased total plant dry matter. In a small repeat experiment, a mixture of the four *Rhizobium* sp. strains also gave lower growth than the control. These results indicate that the *Ensifer meliloti* strains are effective nodulators and N_2 fixers on lucerne but the *Rhizobium* sp. strains are not. Also, it seems likely that competition between *Ensifer meliloti* in the inoculum with less effective indigenous soil rhizobial strains for nodulation can reduce the efficiency of the inoculum and this is likely to reduce lucerne yield. Further genotype work is required to more fully characterise the *Rhizobium* sp. that nodulate lucerne and assess their origin and if they produce functional nodules. On finding that the strains of *Rhizobium* sp. gave poor growth in comparison with *Ensifer meliloti* or uninoculated plants, work on *Rhizobium* sp. was not continued after experiment 1 and only *Ensifer meliloti* was used.

In experiments 2 and 3, addition of *E. meliloti* and additional N and P all had significant positive effects on shoot dry weight and total plant dry weight. Addition of P gave greater growth of *Ensifer meliloti* inoculated plants as has been reported previously (Berg *et al.*, 2005; Monaghan *et al.*, 2007; Stancheva *et al.*, 2008). Also, addition of 25kg N/ha did not depress growth of inoculated plants and if anything gave slightly greater growth. Addition of 'starter' N (~20kg N/ha) has been reported previously to improve early legume growth and enhance the nodulation process (Andrews *et al.*, 2009a). It is likely however that high soil N would depress lucerne nodulation and N_2 fixation (Chambers *et al.*, 1980; Pijnenborg *et al.*, 1990). This was not tested in the current study.

Shoot water content changed little with treatment in experiments 1 and 2 but generally, *E. meliloti* inoculation increased growth was associated with reduced S:R. Possibly, the greater photosynthesis and hence carbon available for growth with inoculation allowed greater root growth which would be more limited in slow growing plants. Shoot to root dry weight ratio also decreased with the increased growth associated with increased N supply from 0 to 50kg N/ha in experiment 4. These results indicate that reduced S:R may be a general growth/N availability response but this needs further study.

In experiments 2 and 3, the data also shows that sowing time has a strong impact on dry matter yield of lucerne. For the first cutting when sowing in August, the total mean for shoot DM reached 191.1g/m^2 in 9 weeks growing in experiment 1. However, the total mean for shoot DM for experiment 2 when sowing in November, only reached 142.67g/m^2. This could be explained by consideration of the physiology of lucerne as a long day plant: spring sowings in New Zealand result in the earliest forage production and the greatest daily growth rate of the seedling crop. Likewise, sowing in summer reduces the yield of lucerne (Wynn-Williams, 1982; Teixeira *et al.*, 2011).

In experiment 2, crude protein, dry matter digestibility and metabolisable energy were not affected by treatment. For the quality measurements, shoot CP, DMD and ME remained consistently above 23%, 69% and 10.7 MJ/kg DM, respectively. This indicates that the feed value of the seedling crops was of high quality at the end of the establishment cycle (Moot *et al.*, 2003; Brown *et al.*, 2005b; Moot *et al.*, 2008). This result agrees with the findings of Washko and Price (1970) where low rates of 22kg N/ha did not affect yield, CP, DMD of lucerne.

In experiment 4, plants relying on N_2 fixation had around 90.8% and 89.3% for shoot and total plant dry matter growth, respectively of plants on optimum soil N. Greater growth with soil N than N_2 fixation has been reported for many other crop legumes (Carlsson and Huss-Danell, 2003; Andrews *et al.*, 2007; 2009a; Lucas *et al.*, 2010; Lowther and Kerr, 2011). Nevertheless, 90% of potential growth of lucerne with N_2 fixation is very high indicating that there is little benefit of adding N to lucerne.

Chapter 4. INTERACTIONS BETWEEN PHOSPHORUS, RHIZOBIA AND MYCORRHIZA ON LUCERNE

4.1 Introduction

Often in agricultural soils, low P availability limits crop growth and inorganic P fertiliser is applied to obtain adequate crop yields. For legumes the lack of soluble P is often a critical limiting factor because it affects not only plant growth but also nodulation and symbiotic nitrogen fixation (Schreven, 1950; Gates and Wilson, 1974; Tinker, 1975; Mosse, 1978).

About 80% of terrestrial plant species including most crop species and lucerne are capable of forming a symbiotic relationship with vesicular arbuscular mycorrhizas (VAM) (Liu *et al.*, 2000; Smith *et al.*, 2001; Bowman *et al.*, 2002; Liu *et al.*, 2004; Asghari *et al.*, 2005; Smith *et al.*, 2011). There is strong evidence to show that mycorrhizal colonization can be of great benefit to plants especially in relation to increased P uptake but that effects of colonization vary markedly depending on environmental conditions and species of both host plant and fungus (Smith and Read, 2008).

Research in recent years has established that under certain conditions, in particular low P soils, use of VAM inoculum can stimulate phosphate uptake and plant growth (Azcon-Aguilar and Barea, 1981; Smith and Read, 2008; Smith *et al.*, 2011). Thus, mycorrhizal symbiosis is a promising cooperation to help plants, particularly in phosphate deficient soils. There are several reports that VAM inoculum can improve nodulation, nitrogen fixation and growth of legumes (Azcón *et al.*, 1991; Barea *et al.*, 2002; Vázquez *et al.*, 2002).

In chapter 3, it was shown that under low soil N conditions in pots, application of *Ensifer meliloti* inoculum can increase growth of lucerne. Also, application of P along with *E. meliloti* can further improve growth. In this chapter, the effectiveness of commercial VAM along with rhizobial inoculation on growth of lucerne under different P availability is assessed in the glasshouse and field.

4.2 Materials and methods

4.2.1 Experiment 5: Effects of *E. meliloti* and *Glomus mosseae* commercial inoculum on growth of lucerne under glasshouse conditions

Experiment 5 had four treatments with two commercial inoculum as Nodulaid (Rhizobia – *Ensifer meliloti*) and Mycormax (Mycorrhiza – *Glomus Mosseae* and *Glomus intraradices*) and control with 4 replicates (4 pots) (Figure 4.1). Experiment 5 used the same basal potting mix as in the experiments in chapter 3 with added 16kg P/ha and 25kg N/ha. The trial started on 16th November 2012 with a shoot cutting on 21st January 2013 and another on 14th March 2013. Shoot fresh weight and dry weight were measured at the first harvest. Shoot fresh weight and dry weight and root dry weight were measured at the second harvest.

Bare seed of lucerne (10 plants/plot = 8kg/ha) were mixed well with inoculant as product directions where appropriate, prior to sowing.

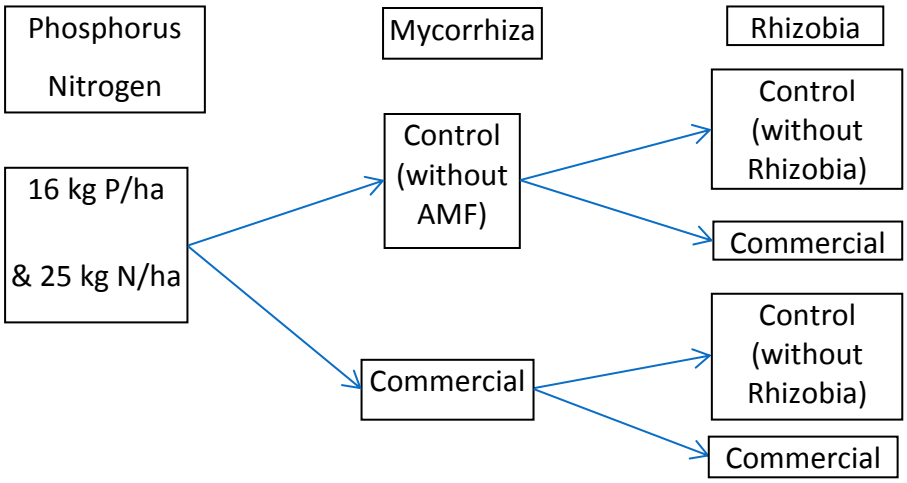

Figure 4.1 Treatments in experiment 5

4.2.2 Experiment 6: Effects of *E. meliloti* and *Glomus mosseae* commercial inoculum and phosphorus on growth of lucerne under field conditions

A field experiment with fully irrigated lucerne 'Stamina 5' (PGG Wrightson) was established as a randomized complete block design with three factors of Rhizobia, Mycorrhiza and Phosphorus, eight treatments, four replicates and two sowing dates (Figure 4.2; Figure 4.3). Plots 10 m^2 (2.1 x 5.0 m) were commenced on the 15th November and 22nd November 2012 for the first and second sowing date, respectively. Bare seed of lucerne (8g/plot = 8kg/ha) was mixed well with inoculant of commercial products as Nodulaid 0.1g/plot (Rhizobia – *Ensifer meliloti*) and Mycormax 4g/plot (Mycorrhiza – *Glomus Mosseae*) based on product directions prior to sowing and P fertilizer (16kg/ha = 16g/plot) was applied by hand directly onto the plots. The eight treatments were:

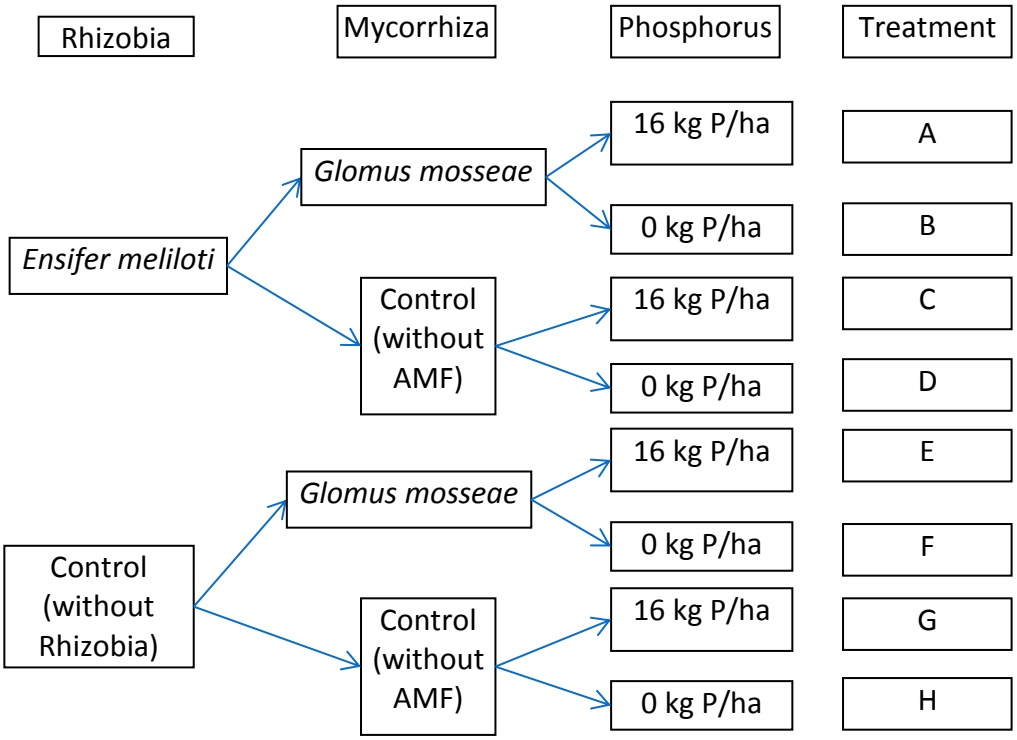

Figure 4.2 Treatments in experiment 6

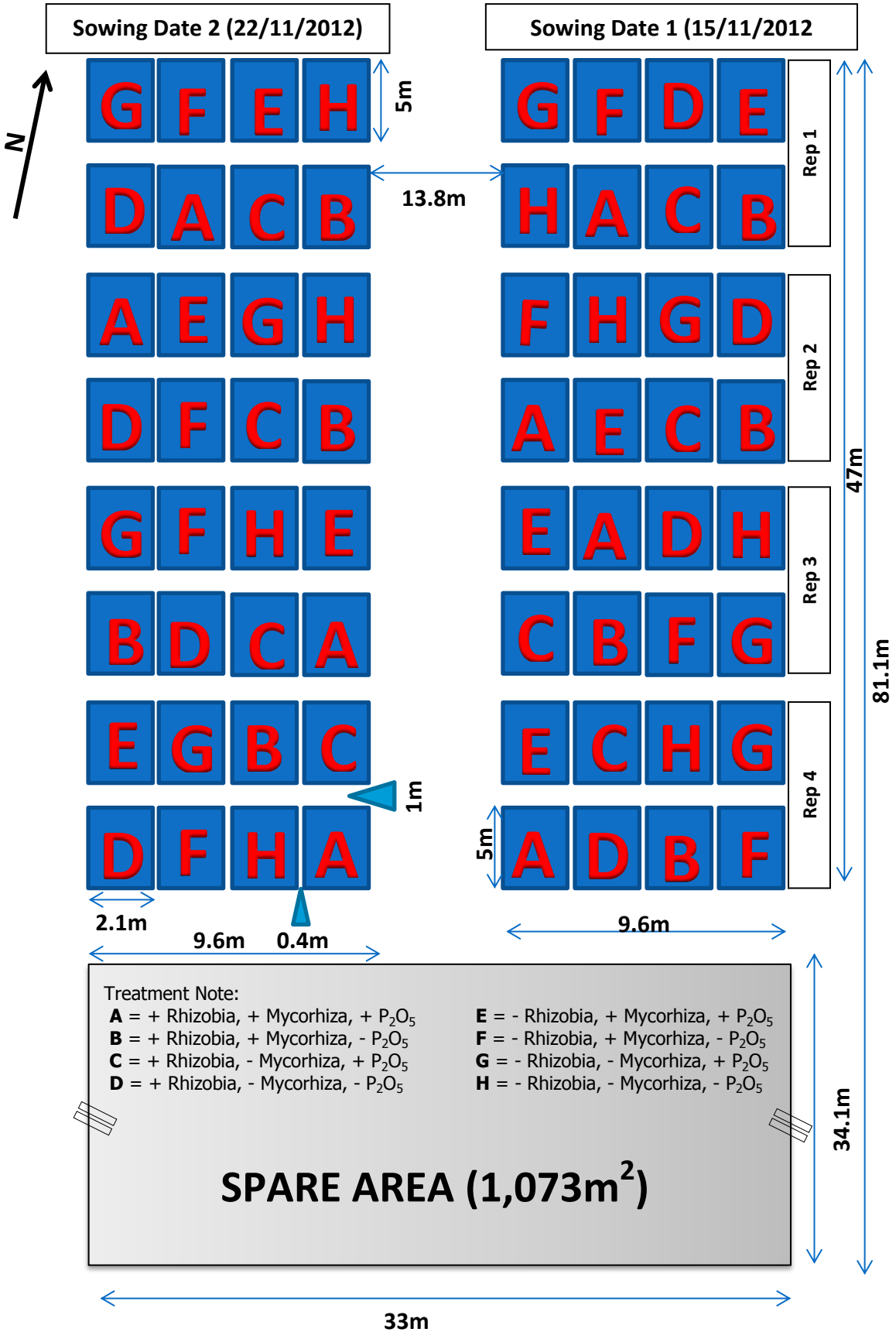

Figure 4.3 Treatments in experiment 6 in the field – Plot H14 FSC.

4.2.3 Site characteristics, meteorological conditions and crop management

The experiment was located at Lincoln University, Canterbury, New Zealand (43°38'S, 172°28'E) within a 0.16 hectare (47 x 33m) area of flat land. The soil is a Wakanui silt loam (*Udic Ustochrept*, (USDA) Soil Taxonomy) with 1.8 – 3.5 m of fine textured material overlying gravels. Soil fertility was evaluated from soil samples which was done from 9 soil cores (30 mm diameter x 150 mm depth) taken randomly from each half (South and North) of the paddock in October 2012. Soil samples were taken and sent to Hill Laboratories and Lincoln University Soil Services Analysis for testing (prior to sowing). Results showed that pH and Ca were in the optimum range for lucerne growth, but K, Mg, Na, S, P and N were low. Phosphorus and N levels, therefore, were suitable for this study with rhizobial and mycorrhizal inoculum. To make the soil favorable for lucerne growth, Potassium Sulphate and Magnesium Oxide were applied at 500 kg/ha and 100kg/ha, respectively (Morton and Roberts, 2012). The soil test results are shown in Table 4.1. Nitrate-N levels were 0.7-1.69 ppm and ammonium-N <0.1 ppm.

Table 4.1 Soil test values from three samples for H14 at Lincoln University, Canterbury, October 2012**.**

Site	Ca	K	Mg	Na	P	$S(SO_4)$	pH
	me/100g				ppm in the soil		
H14	5.0-5.5	0.19-0.22	0.62-0.67	0.07-0.08	11-17	6-8	6.1-6.3
Optimum*	3.0-9.0	0.3-0.5	1.0-1.5	0.2-0.4	20-30	10-12	5.8-6.2

* Hill Laboratories recommendation

The climate in the study area is evenly distributed annual rainfall of about 640 mm and an annual mean temperature of 11.4°C varying from a monthly average of 6.4°C in June to 16.6°C in January. Meteorological data used in the studies were measured at Broadfields Meteorological Station (NIWA, National Institute of Water and Atmospheric Research, New Zealand), which is located 2 km north of the experimental site (Plate 4.1).

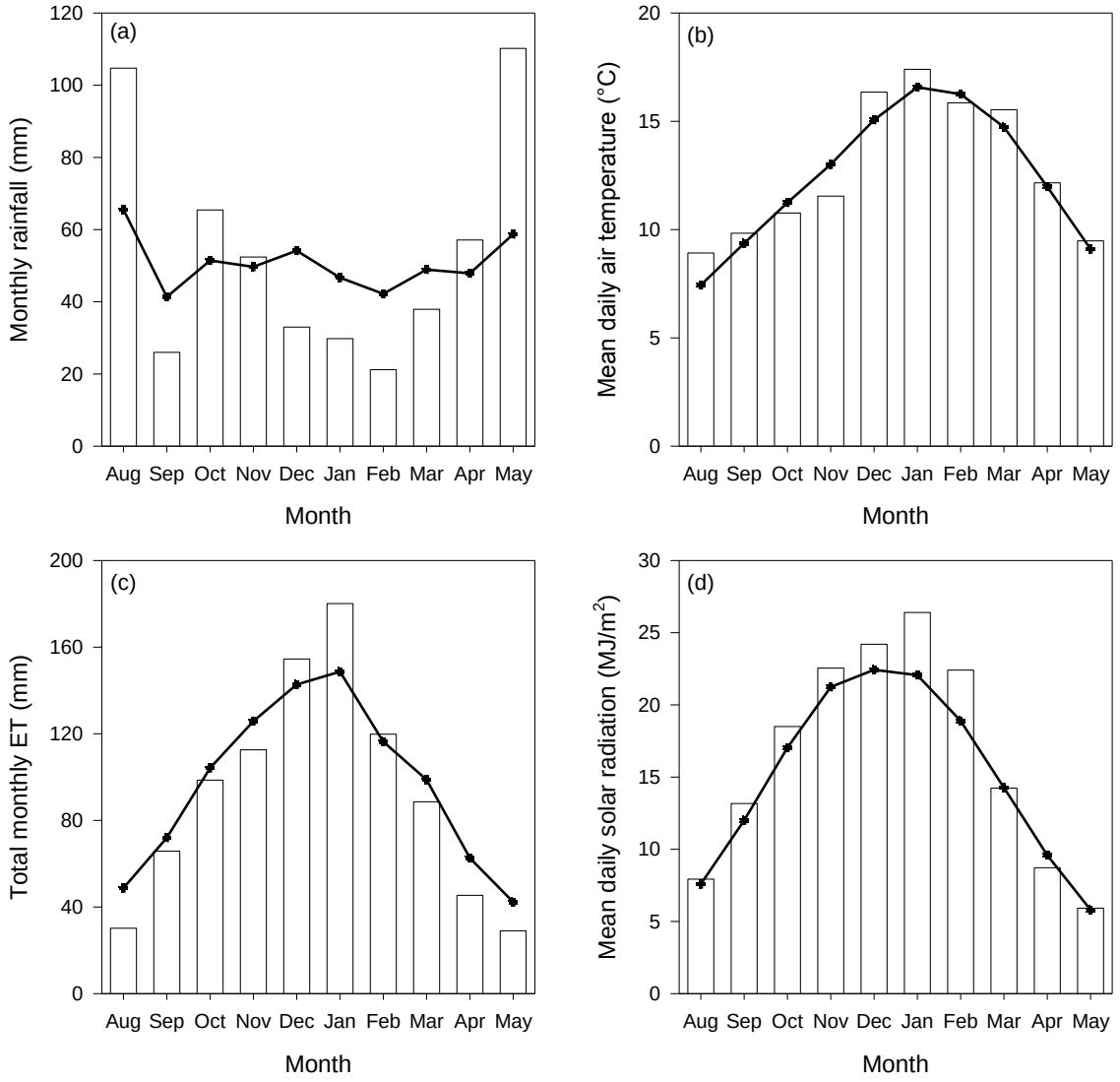

Plate 4.1 Meteorological data from 1 August 2012 to 31 May 2013; long term mean (●) and experimental period (monthly) totals and mean daily (□) taken from Broadfields Meteorological station (NIWA, National Institute of Water and Atmospheric Research, New Zealand), located 2 km north of the experimental site.

The experimental site contained ryegrass (*Lolium perenne*) and barley (*Hordeum vulgare*) in the 2010 and 2011 season, respectively. In May 2011 the area was ploughed and left fallow. From September to October 2012, the paddock was ploughed, roto - crumbled, harrowed and rolled before sowing. The first and second sowing date treatments were 15th November and 22nd November 2012. Seed was sown with a cone seeder (Plate 4.2). Inoculated and un-inoculated 'Stamina' lucerne seeds were sown to 3 mm depth at a rate of 8 kg/ha with 93% germination tested prior to sowing date. After sowing, the paddock was chain harrowed to ensure seed coverage. Chemical control of weeds (spraying Roundup and Spinaker) were used to reduce competition with the establishing lucerne crops and irrigation was applied with the amount from 8-20ml depending on the weather condition to ensure crop growth was not limited by water stress at any time during the experiment (Brown *et al.*, 2006b).

Plate 4.2 Sowing the lucerne seed in the field with cone seeder

4.2.4 Measurements and statistical analyses

Shoot dry matter (DM) measurements were taken on 10th April 2013 (8 weeks growth since last cut), after two cut and carry previously, a Reem Fail mower was used to harvest the above crown height for the field experiment with one straight line cut per plot (height

3cm and width 60cm) (Plate 4.3). All shoot samples were directly weighed in the field by using a Kern Hanging Scale (1 decimal place) and dried in a forced air oven at 65-70°C to constant weight.

Statistical analyses were performed as for experiments in Chapter 3.

Plate 4.3 Samples were harvested in the field by using Reem Flail Mower

4.3 Results

4.3.1 Experiment 5: Effects of *E. meliloti* and *Glomus mosseae* commercial inoculum on growth of lucerne under glasshouse conditions

In experiment 5, both *E. meliloti* and *Glomus mosseae* increased shoot dry weight at the first harvest (Figure 4.4). There was no interaction between *E. meliloti* and *Glomus mosseae.* Shoot dry weight was 22.87% greater with *E. meliloti* and *Glomus mosseae* than the uninoculated control. Shoot water content ranged from 76.75 – 77.38% and was unaffected by treatment.

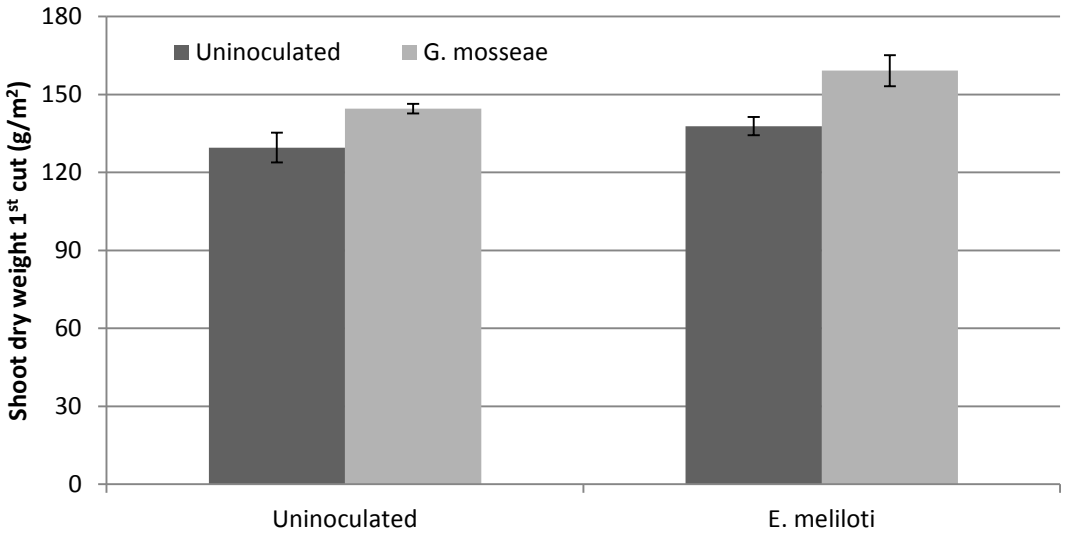

Figure 4.4 Effect of *E. meliloti* and *G. mosseae* inoculum on shoot dry weight of lucerne at first harvest in experiment 5. The vertical bar indicates standard error of mean.

Shoot dry weight and total plant dry weight were greater with *E. meliloti* and *Glomus mosseae* at the second harvest (Figure 4.5). Again there was no interaction between *E. meliloti* and *Glomus mosseae*.

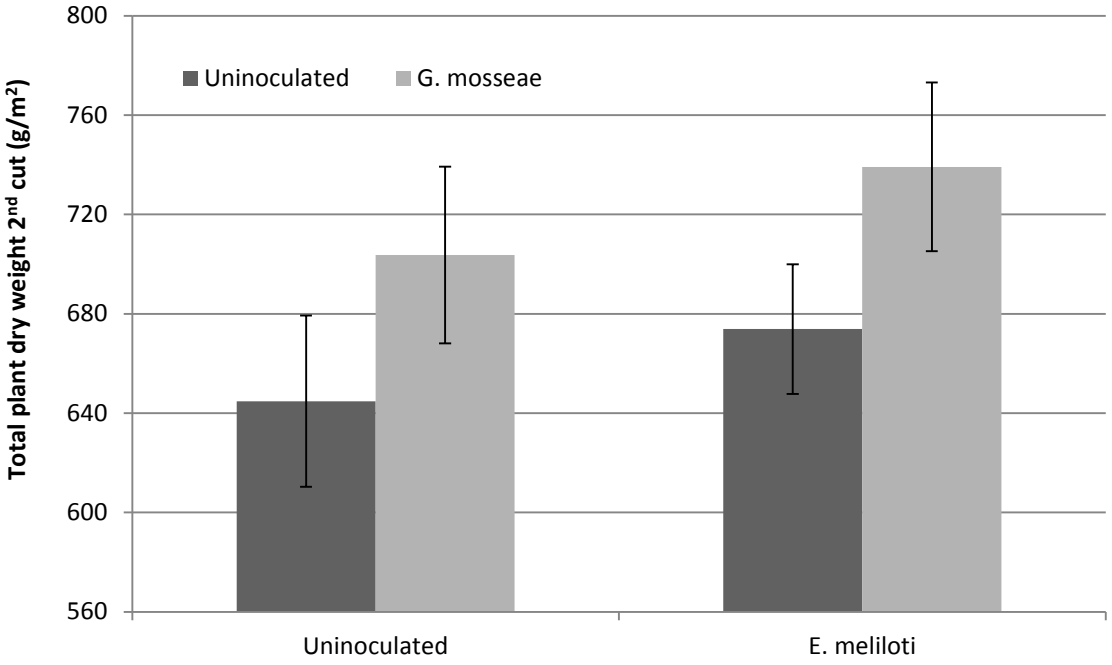

Figure 4.5 Effect of *E. meliloti* and *G. mosseae* inoculum on total plant dry weight of lucerne at second harvest in experiment 5. The vertical bar indicates standard error of mean.

Total plant dry weight was 14.69% greater with 25kg N/ha, *E. meliloti* and 27kg P/ha than with the uninoculated control with 0N and 16 kg P/ha. Shoot water content ranged from 79.52 – 81.05% and was unaffected by treatment. The shoot to root dry weight ratio was also not affected by treatment (Table 4.2).

Table 4.2 Total plant production in 2nd cut (TPDW) (g/m^2), and shoot:root ratio of lucerne with inoculum of *E. meliloti* and *G. mosseae* in experiment 5.

Treatments	Mycorrhiza	Total Plant DM	Shoot:Root
Uninoculated	-[1]	644.8^{c}	1.01^{a}
	+	703.7^{ab}	0.94^{a}
Rhizobium	-	673.9^{bc}	0.95^{a}
	+	739.2^{a}	1.00^{a}
Grand Mean		690	0.98
Pooled SEM		16.79	0.02

[1] '+' and '-' represent with and without uninoculated mycorrhiza
Means with the same letter in column are not significantly different (P<0.05).

4.3.2 Experiment 6: Effects of *E. meliloti* and *Glomus mosseae* commercial inoculum and phosphorus on growth of lucerne under field conditions

Crop dry matter (DM) yields over the measurement period differed between sowing date 1 and sowing date 2 (P<0.001). Yields were greater at the second sowing date (Figure 4.6; Figure 4.7). At both sowing dates, addition of mycorrhizal inoculum or P gave increased dry matter but rhizobial inoculum had no effect. There were no interactions across treatments. Addition of mycorrhiza or P (16 kg/ha) gave similar increases in yield.

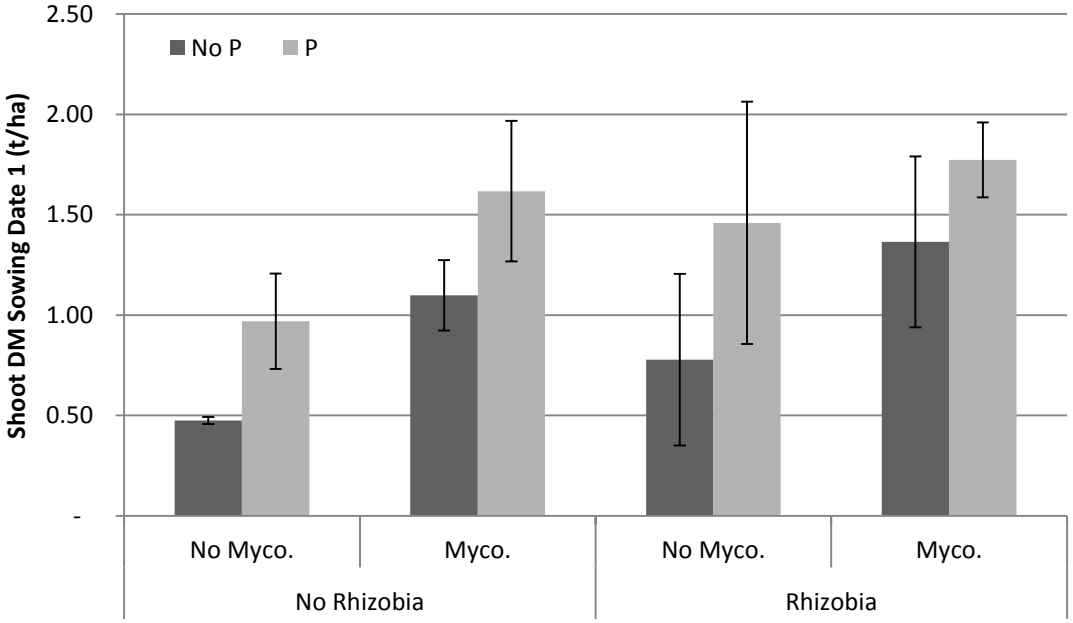

Figure 4.6 Effect of *E. meliloti*, *G. mosseae* and additional P on shoot dry matter of lucerne in sowing date 1, experiment 6. The vertical bar indicates standard error of mean.

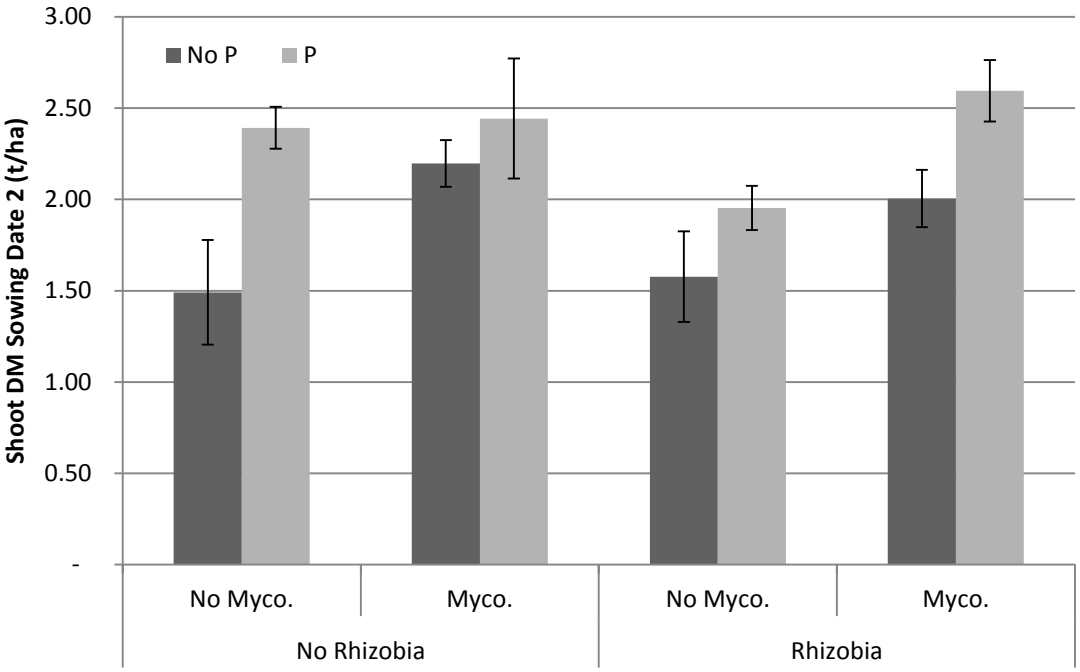

Figure 4.7 Effect of *E. meliloti, G. mosseae* and additional P on shoot dry matter of lucerne in sowing date 2, experiment 6. The vertical bar indicates standard error of mean.

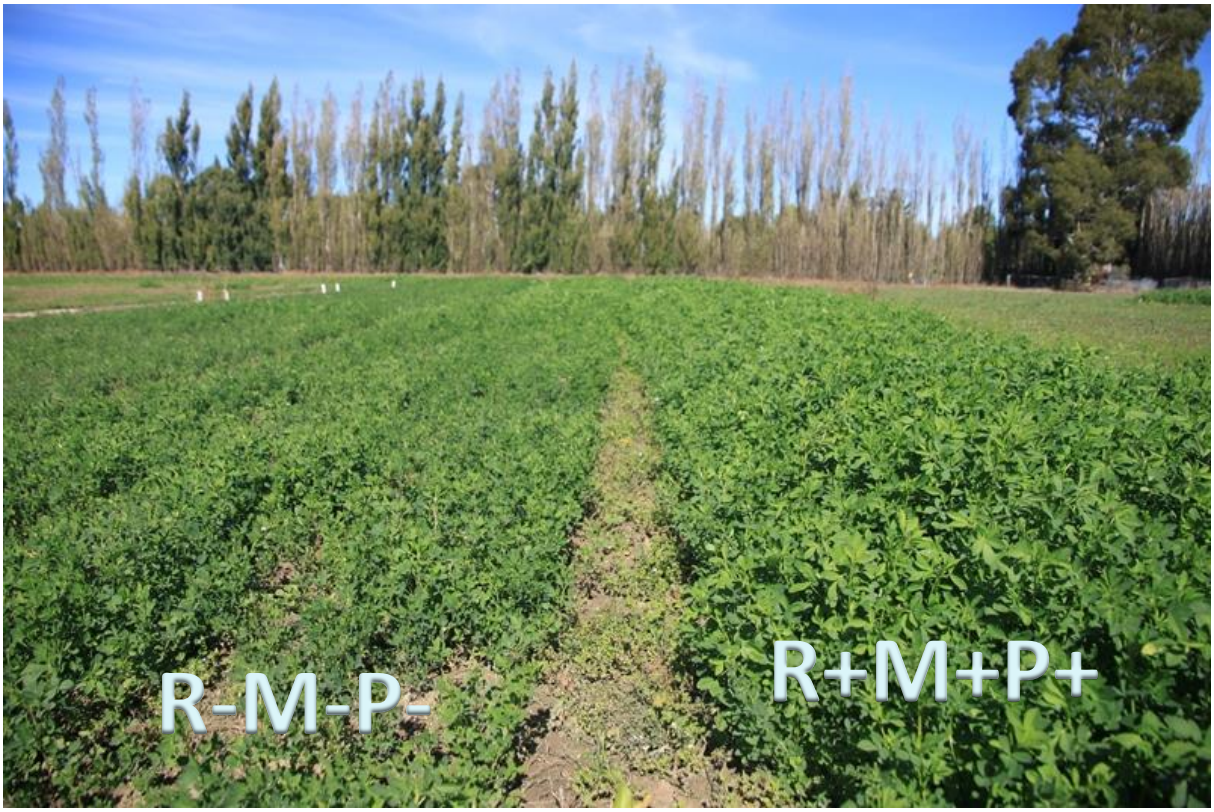

Plate 4.4 A comparison of the minus rhizobial inoculum – minus mycorrhizal inoculum – minus P treatment (R-M-P-) and the plus rhizobial inoculum – plus mycorrhizal inoculum – plus P treatment (R+M+P+).

Plate 4.4 shows a comparison of the minus rhizobial inoculum – minus mycorrhizal inoculum – minus P treatment (R-M-P-) and the plus rhizobial inoculum + plus mycorrhizal inoculum + plus P treatment (R+M+P+). Roots from both treatments were heavily nodulated (Plate 4.5).

Plate 4.5 Roots from both treatments were heavily nodulated

4.4 Discussion

Often in agricultural soils, low P availability limits crop growth and inorganic P fertiliser is applied to obtain adequate crop yields. For legumes, the lack of soluble P is often a critical limiting factor because it affects not only plant growth but also nodulation and symbiotic nitrogen fixation (Schreven, 1950; Gates and Wilson, 1974; Tinker, 1975; Mosse, 1978).

Research in recent years has established that under certain conditions, in particular low P soils, use of VAM inoculum can stimulate phosphate uptake and plant growth (Azcon-Aguilar and Barea, 1981; Smith and Read, 2008; Smith *et al.*, 2011). Thus, mycorrhizal symbiosis has potential to increase crop yields, particularly in phosphate deficient soils. There are several reports that VAM inoculum can improve nodulation, nitrogen fixation and growth of legumes (Azcón *et al.*, 1991; Barea *et al.*, 2002; Vázquez *et al.*, 2002). However, the effects of mycorrhizal inoculant on crop growth are variable and less consistent than with application of P fertilizer (Hodge and Andrews, 2004; Andrews *et al.*, 2010). In general, results indicate that dual inoculation with VAM and rhizobia increases plant growth and N-fixing to a greater extent than inoculation of rhizobia on its own (Ardakani *et al.*, 2009a).

In Chapter 3, it was shown that under low soil N conditions, application of *Ensifer meliloti* inoculum can increase growth of lucerne. Also, application of P along with *E. meliloti* can further improve growth. In this chapter, the effectiveness of commercial VAM along with rhizobial inoculation on growth of lucerne under different P availability was assessed in the glasshouse and field. In addition 'quality' measurements were taken on field samples. In experiment 5, both *E. meliloti* and *Glomus mosseae* increased shoot dry weight at the first harvest and shoot and total plant dry weight at the second harvest. There was no interaction between *E. meliloti* and *Glomus mosseae*. These results show that under N and P limiting conditions, both *E. meliloti* and *Glomus mosseae* can promote growth of lucerne. The mechanism(s) of the growth reponses are not known but it seems likely that *E. meliloti* will work at least in part via increased N acquisition. *Glomus mosseae* could work via increased P uptake but this would need further testing. The shoot water content

and shoot to root dry weight ratio were also not affected by the rhizobial treatments or mycorrhizal treatments.

Under field conditions, crop dry matter (DM) yields over the measurement period differed between sowing date 1 and sowing date 2. Yields were greater at the second sowing date. A possible reason for this is the sowing date 1 was established closer to the boundary of the field which had more transportation and field truck or machines movements which might cause the reduction in DM yields.

At both sowing dates, addition of mycorrhizal inoculum or P gave increased dry matter yield. These results are similar to these obtained under glasshouse conditions. However, in contrast with results in the glasshouse, addition of rhizobial inoculant did not increase yield in the field. A possible reason for this is that there were already high populations of rhizobia in the soil before rhizobial inoculation. This appears to have been the case as plants sampled from plots not inoculated with rhizobia showed substantial nodulation.

Chapter 5. CONCLUSIONS

Alternative strategies are being sought to the application of synthetic N and P fertiliser as a means of combating limiting soil N and P levels in agricultural soils. One alternative method to cope with limiting N is to use a N_2 fixing legume. Use of legume N_2 fixation instead of synthetic N fertiliser would avoid greenhouse gas emissions resulting from N fertiliser production and is cheaper than use of N fertiliser. However, the P requirement of legumes is as great if not greater than that for non-legume crops. There is no microorganism that can add P to agricultural systems, but mycorrhizas can increase the availability of P to crops.

Lucerne can form symbiotic associations with *Ensifer meliloti* rhizobia in root nodules that fix atmosphere N_2. This ability to fix N, can result in very high production of lucerne in low N soils as long as other factors do not severely limit growth. Lucerne can also form mycorrhizal associations with specific fungal species and it has a large and deep taproot that can extract soil water and nutrients from deep layers, and thus has an advantage over many other forage legumes in dry conditions. In New Zealand, lucerne is used for direct grazing and hay making.

The specific objectives of this thesis were to test under different soil N and P availability: 1) The effectiveness of *Ensifer meliloti* and four strains of *Rhizobium* sp. isolated from lucerne in New Zealand on growth of lucerne. 2) The effectiveness of commercial vesicular arbuscular mycorrhizas on growth of lucerne. 3) The interaction between rhizobial and mycorrhizal inoculant on growth of lucerne.

In pots, *Ensifer meliloti* increased, but strains of *Rhizobium* sp. isolated from New Zealand soils in previous studies decreased lucerne total plant dry matter. It seems likely that under field conditions, competition between *Ensifer meliloti* in the inoculum and less effective indigenous soil rhizobial strains for nodulation can reduce the efficiency of the inoculum.

Addition of N and P with *Ensifer meliloti* both increased lucerne total plant dry matter but crude protein, dry matter digestibility and metabolisable energy were not affected by treatment. Feed value of the seedling crops was of high quality with all treatments. Plants relying solely on N_2 fixation had around 90% total plant dry matter growth of plants on optimum soil N indicating that there is little benefit of adding N to lucerne if it is adequately nodulated but P is required in low P soils to achieve high production.

In pots, both *E. meliloti* and mycorrhiza increased shoot and total plant dry weight but there was no interaction between *E. meliloti* and mycorrhiza. These results show that under N and P limiting conditions, both *E. meliloti* and mycorrhiza added together can promote growth of lucerne.

Under field conditions, addition of mycorrhizal inoculum or 16 kg P/ha gave similar increased dry matter yield. These results are similar to these obtained under glasshouse conditions. However, in contrast with results in the glasshouse, addition of rhizobial inoculant did not increase yield in the field. A possible reason for this is that there were already high populations of rhizobia in the soil before rhizobial inoculation. This appears to have been the case, as plants sampled from plots not inoculated with rhizobia exhibited substantial nodulation.

Overall, similar yield increases of lucerne with mycorrhizas and added 16 kg P/ha is an important finding. The potential of mycorrhizas as a mechanism to reduce P inputs into lucerne crops warrants further testing under different soils and agricultural systems in New Zealand. The conditions under which application of commercial rhizobia are likely to be of some advantage require classification.

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