Growth and development of Peas in Response to Different Inoculation Methods and Sowing Dates

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Nguyen Tuan Dung

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Abstract

Peas are the most valuable grain legume exported from New Zealand. The success of a pea crop depends on its capacity to form effective nitrogen-fixing symbioses with root nodule bacteria. *Rhizobium* spp. inoculation of peas has been demonstrated to enhance nodulation, biomass production, harvest index, and nitrogen in the seed. This study evaluated the effects of *Rhizobium* inoculation, nitrogen fertilizer application, and sowing date on the growth, development, final yield and nodulation of garden and field peas grown in Canterbury-New Zealand.

The field experiment used the semi - leafless 'Ashton' garden pea and 'Aragorn' field peas sown on 15 October, 4 November and 30 November 2010 in a Wakanui silt loam soil. Peas were inoculated with either a peat based inoculum or ALOSCA® granules or received 50 kg N ha^{-1} in the form of urea or were un-inoculated as the control.

In most cases *Rhizobium* inoculation and nitrogen fertilizer application had no effect on total dry matter (TDM) accumulation. The accumulated DM of 'Ashton' in the field experiment increased with each delay in sowing date. The DM yield of 'Ashton' from the 15 October sowing was 6.34 t ha^{-1} which was 1.37 t ha^{-1} lower than from the 30 November sowing. The difference resulted from less solar radiation interception due to the green area index (GAI) being below the critical value for most of the season for the early sown crop. There was no difference in TDM accumulation of 'Aragorn' among sowing dates.

In the field experiment, *Rhizobium* inoculation and nitrogen fertilizer had no effect on final seed yield. The highest yield of 4.08 t ha^{-1} was obtained when field peas were sown on 15 October. The final seed yield of garden pea increased as sowing was delayed from 15 October (3.37 t ha^{-1}) to 30 November (3.91 t ha^{-1}). Both cultivars are determinate in their growth habit so their development occurred at a predetermined time with the cessation of leaf production at flowering. Therefore the yield potential in these pea crops depends on

canopy growth rate, green area index (GAI) and consequently the quantity of solar radiation intercepted. Slow leaf appearance (long phyllochron) and therefore canopy development due to low temperatures during early vegetative development resulted in a GAI at flowering for 'Ashton' of only 2.4 when sown on 15 October. This was insufficient to intercept 95% of the photosynthetically active radiation and contributed to the lower seed yield of 'Ashton' in sowing 1 than in sowing 3.

Under these field conditions, *Rhizobium* inoculation treatments were superior to the nitrogen treatment and control in nodulation induction. They gave higher nodulation scores compared with the nitrogen and the control treatment. However, the overall response of peas to *Rhizobium* inoculation was poor with a mean nodulation score ≤ 3 which is considered unsatisfactory. The November sowing, due to a more favourable soil temperature resulted in greater nodulation than in the October sowing.

Overall the results suggest that commercial inoculation of *Rhizobium* to peas did not bring benefits for nodulation, growth or development and thus cannot be recommended. To achieve a final seed yield over 4 t ha^{-1}, to ensure economic viability in New Zealand, a higher sowing rate to achieve at least 100 plants m^{-2} is recommended, particularly for early sown crops.

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Chapter 1 Introduction

Despite a slowing in the growth of the world population from 1.26% per annum from 1996 - 2005 to a predicted 1.10% from 2006 - 2015, the increment continues to be large. It is predicted that there will be an average of 50 - 70 million extra people annually until the 2030s (FAO[a], 2008). As a result, to feed the increased human population, global food production needs to be doubled by 2050 (FAO[b], 2008). In intensive agricultural production, application of fertilizer to supply nutrient elements to plants is important and has become a common practice in farming systems to guarantee final production.

World fertilizer consumption has grown annually by 1.7% from 2007 2008 to 2011 2012. This is equivalent to an increase of 15 million t (FAO[a], 2008). Fertilizers have boosted crop yields, but intensive agricultural systems have a negative effect on the atmospheric and aquatic environments (Jensen and Hauggaard-Nielsen, 2003). In addition, with increased fertilizer prices, in recent years, food prices have also risen (FAO[c], 2008). As world non-renewable resources are depleted by human exploitation, the use of biological energy, in all production sectors, is an important method of maintaining sustainable human development. In agriculture, soil nitrogen (N) availability is a major factor limiting crop yield (Date, 2000). In many agricultural cropping systems, biological N fixing crops contribute substantial inputs of N to the soil. Symbiotic N fixation is renewable and biological N fixation is considered beneficial in:

(1) nitrogen cycling,
(2) restriction of ammonia volatilisation, N_2O emission, NO_3^- leaching,
(3) improving soil structure, erosion protection and giving greater biological diversity (Jensen and Hauggaard-Nielsen, 2003).

Moreover, biological N fixation requires less fossil energy and causes less environmental pollution than inorganic fertilizers (Erman *et al*., 2009a). For these reasons, taking advantage of the ability of legumes to fix atmospheric N can assist sustainability and retain productivity of farming systems in the long term (Jensen and Hauggaard-Nielsen, 2003).

Grain legumes are an important component in arable farming systems in many regions of the world because of the financial return from harvested seed and the fixed N from crop

residues. They are also beneficial in the control of diseases and weeds in crop rotations (Armstrong *et al*., 1994a). The total area cultivated in grain legume occupies about 0.6% of the world's arable land (FAOSTAT, 2009). Peas (*Pisum sativum* L.) belong to the family Leguminosae which is comprised of three subfamilies and approximately 15,000 species that exhibit diverse morphology, habitat and ecology (Denarie *et al*., 1992). Peas are an important crop because of their diversity of utilisation and extensive production area (Boros and Wawer, 2009). There are about 1,000 cultivars of peas grown throughout the world (Cousin, 1997). Pea plants were first grown in the Mediterranean region in 7000 B.C. to provide food for humans and animal feed. From this origin, peas have spread over most temperate regions (McPhee, 2004) and are now grown for human consumption and for hay, or silage to support animal production (Uzun *et al*., 2005). The total cultivated dry pea area in the world is about 6.2 M ha with an average yield of 1.68 t ha^{-1} producing an estimated 105 M t. Half of this production is used for livestock feed, and the remaining half for human consumption, mainly in developing countries (Martin-Sanz *et al*., 2011). Green peas are grown on 2.1 M ha which produce 16 M t (FAOSTAT, 2009). Peas are an excellent source of protein, fibre, minerals and vitamins (McPhee, 2004; Corre-Hellou and Crozat, 2005).

The protein content of wrinkled-seeded pea cultivars is 26 -33 % while the protein in their smooth seeded counterparts is 23 - 31% (Cousin, 1997). Pea seed is a source of vitamins A, B, C and contains 35 - 40% starch, 4 - 7% fibre and relatively high levels of lysine. This makes it an appropriate dietary complement to cereals (Gul *et al*., 2006; Dhama *et al*., 2010) addition to their ability to fix atmospheric N, peas enhance soil structure, and provide breaks for disease control which means they have an important role in modern agricultural systems (McPhee, 2004; Martin *et al*., 2008).

Despite the potential for pea crops in agriculture, they still face challenges due to competition from weeds, insect attack, disease incidence, instability of productivity and a lack of successful nodulation (Date, 2000; Lemerle *et al.*, 2006; Martin-Sanz *et al*., 2011).

The growth and final yield of pea plants are dependent on the formation of nodules and nitrogen fixation by *Rhizobium leguminosarum* bv. *viciae* (Evans *et al*., 1996). Poor nodulation can lead to unsuccessful establishment of pea plants, or ineffective *Rhizobium* bacteria relationship, and consequently poor atmospheric N fixation. Nodulation failure can

be explained by unfavourable field conditions such as low or high pH, high salinity, high soil nitrate content, low soil temperatures, low soil moisture and lack of essential elements (Evans *et al*., 1989; Begum *et al*., 2001) and the absence of effective strains of *Rhizobium* in the soil (Date, 2000). Therefore, inoculation of legumes with desired efficient strains of *Rhizobium* in the form of commercial inoculant products is a common practice to improve nitrogen fixation and final crop yield (Thies *et al*., 1991).

The ability of pea crops to nodulate is a focus of this thesis. In particular crop growth and development are assessed with or without several commercially available seed inoculants. The aim was to determine the efficiency of two commercial inoculants for pea crops sown at different times throughout the spring in Canterbury. To do this the project was split into three objectives.

1. Quantify the effects of spring sowing date on crop growth and development of two pea cultivars.
2. Evaluate the effect of different methods of inoculation on the growth and development of these crops.
3. Determine the ability of the commercial inoculants to successfully induce pea nodules.

Chapter 2 Literature Review

2.1 Introduction

In New Zealand, farmers grow peas for three main reasons: (1) for their cash return, (2) as a break crop for disease control and (3) to improve soil fertility in cereal rotations (White, 1987). The reported amount of N fixed by peas fluctuates between 17 and 83 kg N ha^{-1} (Askin *et al*., 1985). Peas have been grown in New Zealand since the arrival of European settlers in the 19th century. In the 1960's, the pea cultivation area was 10,000 - 12,000 ha of dry peas, which produced 20,000-25,000 t of peas (Lough, 1987). By the 1970's, the pea area had increased to 20,000 ha which produced over 50,000 t of peas. The present cultivated pea area in New Zealand is still about 20,000 ha (Munakamwe, 2008), with a combined value of over $NZ 100 million. Peas are the most valuable grain legume exported from the country (Hill, 1991; Martin *et al*., 2008).

Garden peas are annual cool weather vegetable plants cultivated for their edible green seed or pods. They are harvested at an immature stage to be eaten fresh, canned, and frozen or for processing (Malecki, 1978; Santalla *et al*., 2001). Garden peas play an important role in horticulture because they have a high protein content, atmospheric N fixation, control grassy weeds and disrupt cereal disease life cycles (McPhee, 2004). They also have the potential to provide premium returns to farmers (Anant *et al.*, 2006). In New Zealand, garden peas are sown at a rate to produce populations of around 80 - 100 plants m^{-2} (Hampton and Scott, 1982).

Field peas are often grown in continuous cropping systems as break crops. They are harvested at physiological maturity providing forage for animal feed (Jensen, 1987b; Cousin, 1997; Borreani *et al*., 2007). Agronomically, field peas can control weeds and improve soil N nutrition for subsequent crops, especially cereals (Rowland *et al*., 1994). Field peas can self-provide from 30 to 80 % of their N demand through biological N fixation (Erman *et al.*, 2009b).

In this field experiment, the agronomic and physiological performance of garden and field pea were evaluated to draw conclusions about the potential for expansion, and the importance of *Rhizobium* inoculation in cropping systems in Canterbury, New Zealand.

2.2 Semi-leafless peas in production

Pisum has different variations in foliar morphology (Armstrong and Pate, 1994) because there are various mutants in pea leaves at the basal, proximal and distal locations (Yaxley *et al*., 2001). These mutants are:

'af' gene which converts leaflets to tendrils and transforms the wild-type compound leaf to 'semi-leafless peas'.

'st' gene which gives reduced stipules, and combined with the 'af' gene results in the transformation of the wild-type compound leaf to 'leafless peas'.

The semi-leafless pea is more promising in production than leafless peas. Leaf area is more evenly distributed along the stem of semi-leafless types resulting in greater light and higher air penetration through the canopy which reduces the incidence of disease (Cousin, 1997; Koivisto *et al*., 2003). The tendrils support improved standing ability which increases lodging resistance (Cousin, 1997). Heath and Hebblethwaite, (1984) showed that semi-leafless peas were equal to conventional types in their photosynthetic efficiency but superior in standing ability. In addition, semi-leafless peas had superior water use efficiency and produced a higher maximum DM under dryland conditions compared with conventional types (Wilson *et al*., 1981).

2.3 Agronomic characteristic of peas

Peas are a cool season crop, suited to production in temperate regions and at higher altitudes or in the cooler season in the warmer regions of the world. Peas require specific conditions to grow well and are sensitive to weed competition, moisture stress, disease incidence and rotation length (Corre-Hellou and Crozat, 2005). A suitable temperature range for plant growth is 7 – 24 $^{\circ}$C but the temperature for optimum yield is 12 – 21 $^{\circ}$C (Duke, 1981). It is reported, in another paper, that the base, optimum and maximum temperature for germination of peas are 0 $^{\circ}$C, 29 $^{\circ}$C and 40 $^{\circ}$C, respectively. For vegetative and reproductive growth stages, a base temperature of 3 $^{\circ}$C, optimum temperature of 28 $^{\circ}$C and maximum temperature of 38 $^{\circ}$C are appropriate. Pea crops require around 770 - 890°C d from sowing to flowering and around 1,370 – 1,450°C d from sowing to physiological maturity. In general, there are many conflicting conclusions of what are the correct base temperature and thermal time requirements of peas. Base temperatures from 0 $^{\circ}$C to 5 $^{\circ}$C

are normally used and the differences in the thermal time requirements, of pea, are mostly derived from using various base temperatures in calculations (Olivier and Annandale, 1998).

Peas are suited to a soil with a pH of 5.5 - 6.7 (Ahmed *et al*., 2007), with adequate organic matter which enhances soil structural stability, water holding capacity and biological activity. In India, an example of a developing country, the combined application of animal manure with fertilizer increases nutrient absorption and final yield of pea crops and improves soil fertility (Datt *et al*., 2003). In developed countries phosphate based fertilizers may be applied on deep, well drained sandy to loamy soils that are most suitable for pea production (White, 1987).

Peas are intolerant of waterlogging (Uzun and Acikgoz, 2009) which can cause a decrease in root mass, root penetration depth, plant height or internode extension, leaf expansion, biomass and chlorophyll content (Belford *et al*., 1980; Przywara and Stepniewski, 1999). The anaerobic conditions result in a decline in stem extension, slow transpiration, closure of stomata, acceleration of foliar senescence and desiccation (Jackson *et al*., 1983; Jackson, 1985). Further, plant nitrogen uptake, from the soil, is inhibited leading to reduced nitrogen concentrations in vegetative and reproductive tissues (Jackson, 1979).

High stable seed yield production is the main objective of pea growers. The growth and development of peas are determined by the interaction of genetic factors, the environment and agricultural practices (Acikgoz *et al*., 2009). However, the agronomic requirements of different pea cultivars are variable and depend on their morphology (Witters, 1973; Alvino and Leone, 1993). Therefore optimal growing conditions should be satisfied to guarantee pea performance.

2.4 Pea sowing date

The time of sowing influences the establishment, growth and development of pea crops (Castillo *et al*., 1992). Sowing time can affect the number of days to germination and flowering, the number and weight of green pods plant^{-1} and seed yield (Gill and Ahmad, 1981). Sowing date determines the amount of radiation and the temperature experienced by the crop. As most cultivars have a similar development response to temperature and they require a certain thermal time to reach maturity, in New Zealand early sown crops in late September and early October usually have the potential for high yield as they

experience cooler temperatures, develop more slowly, grow for more days and intercept more solar radiation. The relationship between dry matter (DM) accumulation and intercepted radiation is described as a simple straight line relationship. It is estimated that about 0.9 g of DM is produced for every MJ m^{-2} of radiation. Therefore, the longer a crop grows the more radiation it can intercept and the higher the yield that can be achieved. In contrast, late sown crops develop faster, have shorter growth periods and intercept less radiation and therefore have lower yield potential (PIDG, 2008). However, an experiment in Bangladesh that analysed the growth and yield of garden peas, as influenced by sowing date, showed that plants sown in late November had a higher leaf area index (LAI) and greater yield than earlier sowing in mid-November (Jamil *et al.*, 2006). In India, an experiment with an early maturing pea variety showed that seed yield, pods $plant^{-1}$, pod length, seeds pod^{-1} from a November sowing were all superior to an October sowing (Randhir *et al*., 1996).

In New Zealand, peas are usually sown in the spring (mid - October), because earlier sowing into cold soils gives poor emergence and a weak crop with little chance of producing a high yield and quality at final harvest (Freeman, 1987). In Australia, peas can be sown as a winter crop and sowing time is used to manage disease incidence and severity. It was demonstrated that the level of disease was reduced by delaying sowing time in May, or June, for 3 - 4 weeks. However, the grain yield was 20 % higher in the earlier sown crop rather than the late sown crop in the absence of disease (McDonald and Peck, 2009). In India, greater grain yield and lower disease incidence was obtained from a November sowing over an October sowing (Rai and Gupta, 2003). The incidence of powdery mildew was highest when plants were sown on 15 October and lowest in a 30 November sowing (Sharma, 2002).

2.5 Response of peas to inoculation and inoculation practices

In agricultural soils, where compatible and effective strains of *Rhizobium* are not present, or when efficient Rhizobial soil populations are low, seed inoculation, with selected strains of *Rhizobium* can provide effective legume N fixation (Date, 2000; Chemining'wa and Vessey, 2006). Soil can also contain indigenous inefficient strains of *Rhizobium.* If these strains compete more successfully than the introduced strains for nodule formation, it can lead to legume production failure (Boonkerd *et al*., 1978; Theis *et al.,* 1991; Evans *et al*.,

1996). Therefore the aim of inoculation is to achieve a high proportion of nodules formed on the target host legume occupied by an efficient strain of *Rhizobium* (Evans *et al*., 1989; Thies *et al*., 2001). In Bangladesh *Rhizobium* inoculation has been reported to supplement up to 80 kg N ha^{-1} and increase the average yield of pea plants over uninoculated plants (Ahmed *et al*., 2007). *Rhizobium* inoculation positively affects plant height, the number of branches, root and shoot dry weight, the number of nodules, seed and biomass yield, the number of pods, the crude protein concentration, and seed P content (Erman, 2009a). In India, inoculation of pea with *Rhizobium leguminosarum* at 20 g kg^{-1} seed increased nodulation and activated the nitrogenase enzyme in the root nodules to fix more atmospheric N. Treated plants grew taller and produced more biomass via branching. The harvest index (HI) was enhanced by an increased number of pods and number of seeds pod^{-1} (Karahne and Singh, 2009). Inoculation of peas may also transform root morphology compared with uninoculated plants. Inoculated peas had thicker primary and lateral roots, a greater stele root diameter but a lower root surface area and shorter lateral roots than control plants. The higher stele:root diameter ratio is expected to increase transport from root to shoot through the xylem (Deanedrummond and Chaffey, 1985).

Inoculants can be applied in many ways such as inoculation on seed surface prior to sowing, pre-inoculation in conjunction with seed coating, soil implanting in the form of solid or liquid inoculant, irrigation water run inoculation, inoculant with furrow irrigation of flood irrigation and post emergence inoculation (Brockwell *et al.,* 1975; Heley *et al*., 1980; Danso *et al*., 1990a; Danso *et al*., 1990b; Hegde and Brahmaprakash, 1992; Brockwell and Bottomley, 1995). Each of these methods has its own advantages (Jensen, 1987a). Inoculation responses are highly variable and are site specific (Date, 2000), due to;

(1) a low effectiveness of the *Rhizobium* strain in the inoculants and,

(2) a lower competitiveness of the bacterial strain in the inoculants than existing soil bacteria (Date, 2000).

In addition, the response of plants to inoculation is affected by many factors such as the presence and quality of indigenous rhizobial populations, soil nutrients, soil chemical and physical properties, and the climatic conditions (Thies *et al*., 1991; Begum *et al*., 2001). High temperatures, before and during sowing, may negatively affect inoculum survival

(Jensen, 1987a). Low rainfall and soil with pH < 5.0 or > 8.5 resulted in poorer persistence of introduced rhizobia than more moderate conditions (Howieson and Ballard, 2004).

To have a nodule occupancy of more than 50 %, requires the number of rhizobia in the applied inoculant to be at least 1,000 times greater than the number of indigenous rhizobia (Thies *et al*., 1991). Under the same type of soil, an increase in the inoculation rate from 3.7×10^6 to 3.7×10^8 raised nodule occupancy by the inoculated strain from 74 to 90 % (Boonkerd *et al*., 1978). In the mid-1990's Brockwell and Bottomley (1995) concluded that most inoculants, in the world, were of relative low quality and about 90 % of all inoculants had no practical effect on legume productivity. Inoculation with rhizobia is only useful in locations where there are no indigenous strains of effective rhizobia in soil or the level of the indigenous population is low and the soil N concentration is lower than legume crop requirement (Thies *et al.,* 1991; Catroux *et al*., 2001). However, technological development and improved quality control have given improved quality, reliability and efficacy of inoculation (Catroux *et al*., 2001). The trend to the use of sterile carriers, liquid inoculants or granule technology may avoid contaminants, increase inoculation rates, enhance inoculant shelf-life, improve survival and multiplication of rhizobia in the soil, and facilitate application by farmers (Fouilleux *et al*., 1996; Catroux *et al*., 2001).

Inoculation of legume seed is a simple, practical, widely used method to ensure effective N fixation (Date, 2000; Ahmed *et al*., 2007; Erman *et al*., 2009a). Peat inoculants have become popular for inoculation because they are easy to produce and apply. Peat inoculants can support a high concentration of rhizobia, up to 10^9 to 10^{10} cell g^{-1} peat (Hartley *et al*., 2005). Further it supports survival on inoculated seed (Date, 2000). However, some countries lack natural peat deposits (Graham-Weiss *et al*., 1987) or peat mines are located in forbidden exploitation areas (Daza *et al*., 2000). In addition, bacterial survival on seed is affected by factors which include desiccation, the toxic nature of seed coat exudates and temperature and these limit the availability of peat inoculants (Albareda *et al*., 2008).

Granular inoculants provide the potential to apply rhizobia with greater ease (Denton *et al*., 2009). Granular inoculants can be applied directly to the soil, adjacent to or under the seed. This method of inoculation has the potential to replace peat slurry inoculation in situations where seed fungicides or insecticides need to be applied (Stephens

and Rask, 2000). Granular inoculation enables higher application rates and the physical separation of rhizobia from the seed coat which may contain potentially toxic chemicals (Date, 2000). In addition, granular inoculants provide a major advance in the flexibility of inoculant application. For example, the requirement that seed is sown immediately after inoculation is unnecessary. Soil applied inoculants may result in higher pea biomass, pea seed yield and seed protein concentration, and greater yield stability compared with seed inoculants (Clayton *et al*., 2004a). ALOSCA® granules are a newly released granular inoculum based on bentonite clay granules. They are promoted as having the ability to carry high numbers of viable rhizobial cells and supporting rhizobia under unfavourable conditions. However, peer reviewed literature to support these claims is scarce. As a granular inoculant applied to the soil, it reportedly protects the bacteria from negative effects of fungicides or insecticides applied to the seed (Kiwiseed, 2010).

Inoculation of garden peas may be an effective cultivation practice. Compared with other treatments such as application of farmyard manure or bio-fertilizer, *Rhizobium* inoculated garden pea 'Azad Pea-3' in India had a maximum nodule weight of 8.5 g plant^{-1} due to increased nodulation and root proliferation (Anant *et al*., 2006). *Rhizobium* inoculation significantly increased the protein content and nodulation of garden peas (Prasad and Prasad, 1999a; Prasad and Prasad, 1999b). *Rhizobium* inoculation gave the same pod yield as a conventional fertilizer application (Anant *et al*., 2006). *Rhizobium* inoculation also enhanced DM accumulation, plant weight, the number of shoots plant^{-1} and the plant nutrient content (Choudhary *et al*., 1982; Kanaujia and Raj, 2003). There is no comparable work published for New Zealand so the impact of the inoculum is unknown.

In field peas, *Rhizobium* inoculation has been shown to affect plant height, the number of branches, root and shoot dry weight, the number of nodules, seed and biomass yield, HI, the number of pods (Erman *et al.*, 2009b), and the seed crude protein content compared with N applied at 20 kg and 60 kg ha^{-1} (Erman *et al*., 2009a). Combined application of *Rhizobium* inoculation and 20 kg N ha^{-1} gave a positive response for all these variables (Erman *et al*., 2009a).

2.6 Total dry matter accumulation in pea

In most grain legumes, DM is accumulated slowly during the early stage of vegetative growth. Thereafter DM accumulation is expressed by a near linear growth model until the beginning of pod establishment (Ayaz, 2001; Munakamwe, 2008). The DM accumulation rate then slows down and eventually reaches zero when maximum TDM is accumulated at plant maturity (White, 1991). Biomass, grain yield and N production of pea crops are variable and determined by genotype and growing conditions (Evans *et al.,* 1989; Acikgoz *et al*., 2009). In the field DM accumulation is often increased at high plant populations up to 400 plants m^{-2}. In New Zealand, as population was increased from 10, 100, 200 to 400 plants m^{-2} peas produced from 2.92 - 6.70 t DM ha^{-1} (Ayaz *et al*., 1999). At high populations, canopy closure was more rapid which resulted in greater interception of incoming solar radiation (McKenzie and Hill, 1991; Ayaz *et al*., 1999). In addition, there was a positive correlation between plant height and biological yield of pea (Dhama *et al.*, 2010) because taller plants exposed a greater leaf area for light interception (Hiebsch *et al*., 1990).

The effect of N on DM accumulation of peas is variable. In Canada N application significantly increased forage yield (Sosulski and Buchan, 1978). In contrast in New Zealand N application had no influence on shoot DM accumulation (Gunawardena *et al*., 1997). The soil N level, at establishment, and N mineralisation rates during the growing season will influence the impact of nodulation.

2.7 Harvest index and grain yield

The HI is the economic yield of a crop expressed as a decimal fraction of total, above ground, biological yield (Hay, 1995). The HI, and grain yield, of grain legumes is more variable than in many other crops (Ayaz *et al.*, 2004). Pea crops have poor yield stability both within and between sites and seasons. Harvest index variation has been identified as the main contributor to yield instability (Ayaz *et al*., 2004). A range from 0.00 to 0.74 has been reported in grain legumes (Husain *et al.*, 1988). Harvest index of peas differs among cultivars and with growing conditions (Ambrose and Hedley, 1984; Srivastava and Asthana, 1994). Moot and McNeil (1995), in a single plant study, reported that plant HI of different pea cultivars ranged from 0.53 - 0.62. Ambrose (1984) and Moot (1995) concluded that plants which form a more uniform population will have a higher plant HI although single plants may perform poorly (Ambrose and Hedley, 1984; Moot and McNeil, 1995). Chandra

and Polisetty, (1998) found that early flowering cultivars had a higher HI than medium and late flowering cultivars. The HI of the early variety was 44 % against 42 % and 36 % for medium and late flowering cultivars respectively. The reason for the higher HI in the early flowering variety was greater translocation of photosynthate to reproductive organs and lower photorespiration compared with medium and late flowering varieties.

Final seed yield of peas, in New Zealand, was reported to range from 2.59 - 5.5 t ha^{-1} depending on growing conditions such as the timing and intensity of drought (Martin and Jamieson, 1996). The yield of peas depends on many genetic, agro-ecological factors, pest and disease incidence and management practices (Jasper *et al*., 2000; Tripathi and Mithlesh, 2006; Prusinski *et al*., 2008; Rapcan *et al*., 2010). A positive association was demonstrated between grain yield and days to 50 % flowering, days to maturity, plant height, number of pods $plant^{-1}$, number of seeds $plant^{-1}$ and pod length by Dhama *et al*. (2010) and Rapcan *et al*. (2010).

2.8 Yield components

Pea seed yield can be described by four yield components, plants m^{-2}, number of pods $plant^{-1}$, seeds pod^{-1}, and the mean seed weight (Wilson, 1987).

2.8.1 Plants unit area^{-1}

Plant population explains from 68 - 70 % of the variation in pea yield (Mera, 1989). The yield of pea crops normally increases, with increasing population, until it reaches an optimum density. Moot (1995) showed seed yield of pea crops doubled from 350 g m^{-2} at 9 plants m^{-2} to 675 g m^{-2} at 400 plants m^{-2} (Moot and McNeil, 1995). However once the plant population exceeds the optimum it can lead to a progressive decrease in yield. The optimum population for peas is variable and depends on the cultivar and the growing conditions (Wilson, 1987). For example, green pea yield under dryland conditions did not increase beyond 83 plants m^{-2} but under irrigation yield increased up to 135 plants m^{-2}. In a later year, under both dryland and irrigated conditions, higher yield was recorded at 101 plants m^{-2} compared to 65 plants m^{-2} (White *et al*., 1982).

Semi-leafless peas can be sown at target variable population of 80 - 140 plants m^{-2}. The increase in plant population decreased standing ability but prolonged the reproductive phase and increased seed yield. It is suggested that a plant population 100 - 120 plants m^{-2} is

optimum for semi-leafless peas (Sawicki *et al*., 2000). Different semi-leafless pea cultivars require different plant populations to maximise their yield. However, a plant population from 70 - 140 plants m^{-2} is believed to be satisfactory to maintain maximum yields due to the ability of peas to compensate density decline by producing increased pods $plant^{-1}$. At below 70 plants m^{-2}, the risk of yield reduction is increased (Heath *et al*., 1991). In this experiment, a target plant population of 100 plants m^{-2} was chosen for both the garden and the field pea cultivars.

2.8.2 Pods Plant^{-1}

The number of pods $plant^{-1}$ and plants unit $area^{-1}$ have been demonstrated to be the yield components most strongly correlated to pea seed yield (Hardwick *et al*., 1979; White, 1987; French, 1990). The number of pods $plant^{-1}$ is variable and depends on cultivar. Many older cultivars bear a single pod $node^{-1}$ whereas most current cultivars bear two pods $node^{-1}$ and some vining pea cultivars produce up to five pods $node^{-1}$ and this means that the number of pods $plant^{-1}$ was also increased (Knott, 1987).

2.8.3 Seeds pod^{-1}

There are normally 5 - 6 seeds contained in a pea pod but this depends on the cultivar and the growing conditions (Knott, 1987). Atmospheric CO_2 enrichment or supplementary lighting will reduce seed abortion (Jeuffroy and Chabanet, 1994). In contrast, elevated temperatures at the seed development stage, shading, an increase in plant density, and defoliation will increase the frequency of seed abortion and as a result will decrease seed number $plant^{-1}$. In addition, seed number pod^{-1} is correlated with pod growth in particular with early pod elongation (Jeuffroy *et al*., 1990; Jeuffroy and Chabanet, 1994) . Irrigation at flowering, and pod filling, can result in an 84 greater green pea yield and from a 33 % to 78 % greater seed yield. These increases were the result of increases in pods $plant^{-1}$, seeds pod^{-1} and mean seed weight (White *et al*., 1982). Generally, water stress at any stage results in a proportional decrease in yield (Wilson 1987).

2.8.4 Mean seed weight

Seed weight is the most stable yield component (Littleton *et al*., 1979; Saxena, 1980; Saxena and Sheldrake, 1980; Saxena *et al*., 1983). Pea seeds differ in size and shape, with size ranging from about 90 mg $seed^{-1}$ to 400 mg $seed^{-1}$ (Knott, 1987). The mean seed weight

of peas depends mostly on cultivar. However, in an experiment in New Zealand, Castillo *et al*. (1994) found that November sown plants had a heavier seed weight than December sown peas.

2.9 Genetic identification of *Rhizobium* in nodules based on molecular approaches

Molecular based techniques are important for the characterization of bacterial isolates because the techniques can now solve many of the shortcomings of other approaches such as the need to culture *Rhizobium* to perform analyses, the ability to follow a single strains or a few strains over time in the field, labour intensity and the lack of ability to characterise the nature of indigenous populations of *Rhizobium* (Thies *et al*., 2001; Vachot-Griffin and Thies, 2005). The value of molecular approaches is in their relative ease, and their ability to provide information and discrimination about all members of a given community. Fundamental to the method is the ability to define the specific nucleic acid sequences of a particular microorganism or group of related microorganisms (Barry *et al*., 1991). The process of characterisation of bacteria, generally and *Rhizobium* particularly usually starts with extraction of nucleic acids (DNA/RNA) from cells by various methods, followed by the use of this DNA in polymerase chain reaction (PCR) amplification. The PCR products are then resolved by electrophoresis and the result patterns used to distinguish the species of bacteria (Jensen *et al*., 1993; Thies *et al*., 2001). The (PCR) amplification of specific DNA sequences has widespread application because it has capacity to amplify unique sequences in the midst of a myriad of DNA sequences. By the design of specific oligonucleotide primers and the ability to repeatedly amplify the specific DNA sequence, the technique can detect and identify specific strains of *Rhizobium* found in the soil and in nodules even when *Rhizobium* are present in low numbers in mixed infections of nodules (Pillai *et al*., 1992).

The PCR consists of three tightly connected processes. These are the separation of a double-stranded DNA template into 2 single strands (denaturation), the hybridisation (annealing) of oligonucleotides (primers) to the template and then elongation of the primer template hybrid by a polymerase enzyme (Thies *et al*., 2001). The polymerase enzyme used to catalyse PCR amplification is the thermo-stable enzyme *Taq* polymerase (Saiki *et al*., 1988). The primers used in PCR are diverse and depend on the potential target genes (Thies *et al*., 2001). In *Rhizobium* studies, the primers may be designed to target specific DNA

fragments such as 16S ribosomal RNA genes, 16S-23S rRNA intergenic spacer regions or genes that regulate nodulation or nitrogen fixation (Dobert *et al*., 1994; Perret and Broughton, 1998; Thies *et al*., 2001). The 16S sequence contains variable regions that are widely used to identify genera and species (Jensen *et al*., 1993). However, among closely related microorganisms the variation observed between the 16S rRNA is small. This resulted in the utilization of 16S/23S ribosomal spacer sequences as the potential target fragments because it has greater variability (Barry *et al*., 1991). On the other hand, repetitive sequences such as the repetitive extragenic palindromic (REP) sequence, enterobacterial repetitive intergenic consensus (ERIC) sequence and interspersed repetitive DNA (BOX) sequence are also the target sequence of primer design in PCR (Hulton *et al*., 1991; Versalovic *et al*., 1991; Martin *et al*., 1992; Niemann *et al*., 1999; Thies *et al*., 2001). All of these primers have been used to obtain PCR-fingerprints which are the foundation to characterise *Rhizobium* isolates at the strain level (Thies *et al*., 2001). The REP sequences locate in the untranslated regions of operons and have been proposed as important in many functions including transcription termination, mRNA stability, and chromosomal domain organization *in vivo*. The ERIC sequences contain a highly conserved central inverted repeat and are located in extragenic regions. It has been demonstrated that the inter-REP or inter-ERIC distances are specific for bacterial species and strains (Versalovic *et al*., 1991).

Amplified PCR products are often visualised under UV by running samples out on an electrophoresis gel and then staining the gel with ethidium bromide. The presence and pattern of DNA bands in the gel matrix help to analyse PCR products. Agarose is the most common medium for electrophoretic separation of medium and large size nucleic acids. Agarose gel, at different concentrations, is used to separate nucleic acids between 0.1 and 70 kb in size. However electrophoretic separation on agarose gel normally gives poor resolution. To achieve higher resolution for electrophoretic separation polyacrylamide gels can also be used (Thies *et al*., 2001).

ERIC sequences are highly conserved in *Rhizobium* and the ERIC PRC method can be used successfully to distinguish and classify even closely related *Rhizobium* strains. This is because patterns of the result of the PCR products after analysis on agarose gels are highly specific for each strain (Debruijn, 1992). Therefore, in this work, ERIC 1 and ERIC 2 primers were used in the PCR reaction to examine the ERIC sequence in the genomes of *Rhizobium*

leguminosarum viciae isolated from the root nodules of pea plants to evaluate the presence of this specific *Rhizobium* strain in the commercial inoculant.

2.10 Summary

The success of pea production is determined by agronomic and physiological factors. Satisfactory nodulation and effective nitrogen fixation to satisfy pea demand as well as improve soil nitrogen status are important. However, in some cases, nodulation of peas is poor and *Rhizobium* inoculant application is believed to promote nodule establishment in peas. In New Zealand, there is little research on *Rhizobium* inoculation effects on pea nodulation and this is the main focus of the thesis. The thesis examined the growth, development and nodulation of peas through measurement and evaluation related variables such as plant height, LAI, thermal time, nodulation, nodule occupancy, DM accumulation, yield components and final seed yield. It aims to provide farmer information about the response of 'Ashton' garden and 'Aragorn' field pea to different sowing dates and the effectiveness of method of *Rhizobium* inoculant application.

Chapter 3 Materials and Methods for Field Trial

3.1 Field Trial

3.1.1 Location and paddock history

The experiment was conducted in Iversen field of the Field Service Centre, Lincoln University, Christchurch, New Zealand (44° 38' S, 172° 28' E) at 7 m above sea level. The experiment was established in the 2010 - 2011 growing season in a uniform area of 1.3 ha of Paddock I3 which was previously under arable cultivation. Mixed pasture which included perennial ryegrass (*Lolium perenne* L.), white clover (*Trifolium repens* L.) and chicory (*Cichorium intybus* L.*)* were grown from 2004 - 2006. Oats (*Avena sativa* L.), brassica (*Brassica rapa* L.*)*, barley (*Hordeum vulgare* L.*)* and ryegrass were grown in the paddock in 2007, 2008, 2009 and 2010, respectively.

3.1.2 Soil type

The soil type is a Wakanui silt loam (USDA classification) which is imperfectly drained and formed from fine alluvium and indurated sandstone and siltstone (Yeates *et al*., 1998). Plots were 4.2 x 5 m and were separated by 6 m to facilitate the operation of an Öyjord cone seeder and other machines. A Ministry of Agriculture and Forestry (MAF) quick soil test was taken at sowing to obtain details of soil fertility (Table 3.1). At the flowering stage of every sowing, soil samples were collected to determine soil nitrogen content. Each sample from every treatment consisted of a bulked sample of 16 soil cores taken from depths of 0 - 0.15 m across the field. The soil test result showed that nitrogen content in the experimental area ranged from 43 to 58 kg ha^{-1}.

Table 3.1: MAF soil quick test for paddock I3 in October, 2010 , Field Service Centre, Lincoln University.

pH	P (µg ml^{-1})	Ca	Mg	K	S (µg g^{-1})
6.4	20	12	24	10	2

Ca, Mg, K as mg/g of soil

Land was prepared using conventional cultivation of ploughing, rolling, and harrowing with fertilizer being applied in October, 2010. Soil was tilled to a depth of 0.25 m.

Superphosphate (0:9:0) was applied at 300 kg ha^{-1} before sowing. Glyphosate at 3 l ha^{-1} and Treflan (Trifluralin 5%) at 3 l ha^{-1} were applied on 28 September 2010 and 13 October 2010 to control weeds before emergence. Alto-S (100 g l^{-1} Cyproconazole; 96 g l^{-1} 2-pyrrolidinone, 1 methyl) at 250 ml ha^{-1} was applied on 13 January 2011 to combat powdery mildew (*Erysiphe* spp). No insect or further weed control was required throughout the duration of the experiment.

3.2 Experimental design and methods

The experiment was a split plot design with 24 treatments and four replicates. Sowing dates (15 October; 4 November; and 30 November 2010) were main-plots. The combination of the control, N treatment (50 kg ha^{-1}) and inoculation (peat or ALOSCA ® granules) and the two semi-leafless pea cultivars ('Aragorn' or 'Ashton') gave a total of 96 sub plots.

For the pea cultivars used in this experiment, the early maturity 'Ashton' garden pea has a large range of recommended sowing times from late July through to early December. The mid-season 'Aragorn' field pea has a shorter range of recommended sowing time from September to the end of October (PIDG, 2008). Three different sowing dates: 15 October, 4 November, and 30 November were chosen to evaluate the response of these two different cultivars to sowing date. These were all within the recommended sowing time for 'Ashton' but the latter two were outside the recommended range for 'Aragorn'.

For the *Rhizobium* inoculant used in this experiment, peat inoculant applied to the seed and ALOSCA® granules, applied to soil prior to sowing, were used to evaluate their effect on the nodulation, growth and yield of garden and field peas.

One week before the first sowing, five Petri dishes with 25 normal seeds (undamaged, typical shape, green in colour, normal size) from each pea cultivar were incubated at 23 $^{\circ}C$ to determine seed germination. In addition, the 1,000 seed weight (TSW) of each cultivar was determined. The results were 194 g for 'Aragorn' and 186 g for 'Ashton'. The results of the germination tests and the TSW were used to calculate the appropriate quantity of seed to be sown to obtain a target plant population of 100 plants m^{-2} in each plot. A day before sowing, 25 kg of N ha^{-1}, as urea, was applied by hand onto the 50 kg N treatment plots. A further 25 kg of N ha^{-1} was applied at early flowering. For the peat

inoculation treatment the peat inoculant was mixed in water and then with the seed at 2.5 g of peat inoculant kg^{-1} of seed. After mixing seed with the slurry it was spread out on clean plastic bags and dried at room temperature for 8 hours in the Field Service Centre crop laboratory. The ALOSCA® granules at 10 kg ha^{-1} were mixed with the seed by shaking in a clean plastic bag and were then applied directly to soil simultaneously with the seed at sowing using an Öyjord cone seeder.

3.3 Measurements

In this thesis, variables including plant height, leaf appearance, nodulation, TDM accumulated, GAI, number of pods $plant^{-1}$, number of seeds pod^{-1}, mean seed weight, and final seed yield were measured and are discussed as indicators of crop responses to sowing date and inoculation.

3.3.1 Meteorological data

Rainfall (mm), solar radiation (MJ m^{-2} d^{-1}), wind speed (m s^{-1}) and air temperature (°C) were recorded at Broadfields Meteorological Station located 2 km to the north of the site using standard National Institute of Water and Atmospheric Research equipment. Measurements were recorded hourly and calculated to daily values.

The 2010 - 2011 growing season received less rain than normal which was expressed as long-term data (Figure 1). There was only 23.6 mm of rain in October which was about 50 % of the long term average (52.2 mm). Rainfall in December, January and February was about 10 - 20% lower than long term (1975 - 2007) average.

The mean monthly air temperature in October, November and December was warmer than the long term average. December was the hottest month during the growing season with an average of 16.8 °C which was nearly 2 °C warmer than the long term mean (Figure 2).

Solar radiation receipts in the 2010/2011 growing season were higher than the long-term averages in October and November but were lower in December, January and February (Table 3.2).

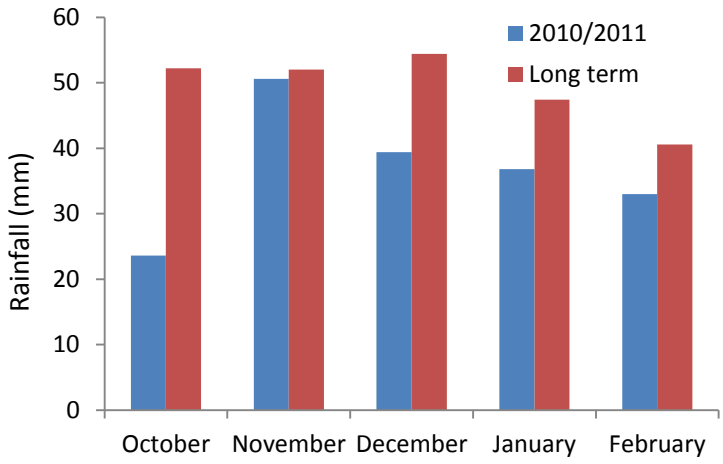

Figure 1: Mean monthly rainfall (mm) at Broadfields Meteorological Station in the 2010/2011 growing season and the long term mean (1975 - 2007).

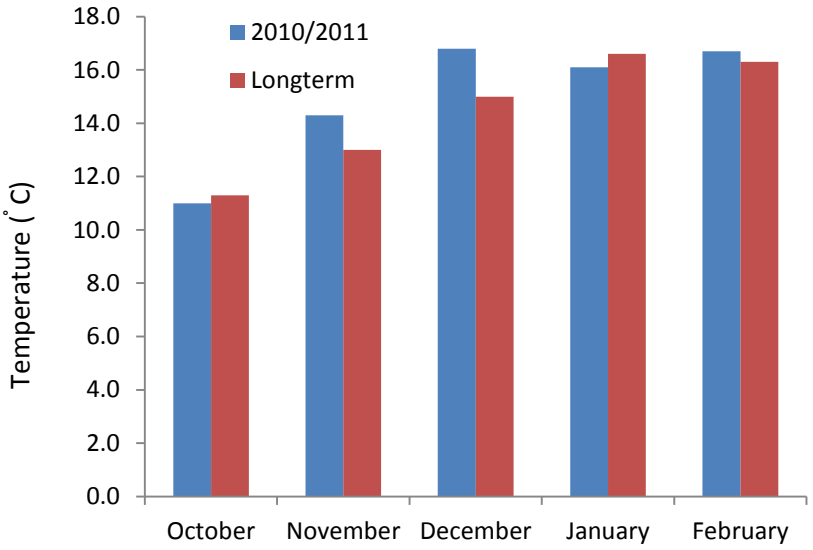

Figure 2: Mean monthly air temperature (˚C) at Broadfields Meteorological Station in the 2010/2011 growing season and the long term (1975 - 2007) mean temperature.

Table 3.2: Mean monthly solar radiation (MJ m^{-2}) at Broadfields Meteorological Station in the 2010/2011 growing season and the long term (1975 - 2007) mean.

Month	2010/2011	Long term (1975-2007)
October	617	557
November	733	697
December	701	739
January	691	727
February	570	625

3.3.2 Seedling emergence

After sowing, as seedlings began to emerge, two 1 m long strips in the middle rows of each plot were marked and observed every 1 to 2 days to collect emergence data (Gan *et al*., 2002). This continued for each sowing until no further plants emerged on at least three consecutive observation days. The results of the emergence observation were used to calculate actual plant population m^{-2} by multiplying the number of plants in the 1 m long strip by 7 which was the number of rows in 1 m of width.

3.3.3 Plant height and green leaf number

Also from the middle of the third row of each plot, 10 plants were marked and the height from soil surface to the top of the plant was measured with a rule every 7 to 10 days. At the same time the number of nodes with green leaves was counted and observations of date of first flower and pod appearance were recorded.

3.3.4 Thermal time accumulation

Thermal time accumulation and the leaf appearance rate, was calculated after an initial test to establish base temperature. There was little difference in the R^2 values (R^2 = 0.91 - 0.92) between a base temperature of 0 °C and 4.5 $^{\circ}$C so 4.5 $^{\circ}$C was used to be consistent with previous reports (Olivier and Annandale, 1998).

Thermal time accumulation from emergence to flowering was calculated as a sum of daily thermal time accumulated (Tt, °C d).

$$Tt = \Sigma(Tmax + Tmin)/2 - Tb$$

'Tt' is set as zero when the mean air temperature below the base temperature 'Tb' of 4.5 °C or above maximum temperature 'Tx' of 38 °C. Thermal time increases linearly between 'Tb' and optimum temperature 'Tm' and decreases linearly between 'Tm' and 'Tx' (Olivier and Annandale, 1998).

3.3.5 Leaf appearance rate (Phyllochron)

Leaf appearance (°C d stem node^{-1}) was calculated as the linear regression between the number of leaves on the stem and thermal time accumulation from emergence to flowering.

3.3.6 Green area index (GAI)

From five weeks after sowing, when the pea plants were over 0.15 m in height, the green area index was measured using a sun scan plant canopy analyser (SS1 Delta-T device, Cambridge, England) every 10 to 14 days. Eight random readings were taken to obtain an average number for each sub-plot.

3.3.7 Dry matter yield

Dry matter production measurements were taken from each sub-plot by hand cutting to ground level a 0.2 m^2 quadrat at 10 to 14 day intervals. Dry matter samples were dried in a forced draft oven (65-70 °C) to constant weight.

3.3.8 Pod characteristics

At maturity (when the leaf canopy started to change to yellow), 10 random plants were cut and were used to determine pod length, the number of pods plant^{-1} and seeds pod^{-1}.

3.3.9 Total biomass, seed yield, harvest index and thousand seed weight

At crop maturity, plots were harvested with hand shears cut to ground level to measure the final total biomass. The peas were harvested at physiological maturity by cutting 2 x 0.5 m^2 quadrats from each plot. Plants were then oven-dried at 70 $^{\circ}$C for at least 48 hours to constant weight for dry weight determination. Plants were threshed to collect the seed and the seed was weighed.

3.3.10 Nodulation assessment

From five weeks after sowing, at two weekly intervals, five plants from the third row of each plot were randomly, and gently, dug out to avoid damaging the root system and losing nodules. The pea plants were transported to the laboratory at the Field Service Centre and the soil was gently removed using clean water. Nodulation assessment was measured on the same day using the classification of Corbin *et al*. (1977).

Table 3.3: Classification of nodulation score of peas grown in Canterbury-New Zealand in the 2010/2011 growing season (From Corbin *et al*., 1977).

Nodulation score	Distribution and number of effective nodules	
	Crown	Elsewhere
0	0	0
0.5	0	1-4
1	0	5-9
1.5	0	≥10
2	Few	0
2.5	Few	Few
3	Many	0
4	Many	Few
5	Many	Many

Effective nodule judged on the basis of nodule size and internal pigmentation; ineffective nodules not considered.

Crown was regarded as the top 50 mm of the root system.

The root system of the pea plants was observed and photographed at the same time as the nodulation assessment was made. Plates 1 - 8 show the pattern of pea root nodule distribution for different nodulation scores (Corbin *et al*., 1977).

Plate 1: The root system of a pea plant with a nodulation score of 0 according to the nodulation scale of Corbin *et al*. (1977).

Plate 2: The root system of a pea plant with a nodulation score of 0.5 according to the nodulation scale of Corbin *et al*. (1977).

Plate 3: The root system of a pea plant with a nodulation score of 1.5 according to the nodulation scale of Corbin *et al*. (1977).

Plate 4: The root system of a pea plant with a nodulation score of 2 according to the nodulation scale of Corbin *et al*. (1977).

Plate 5: The root system of a pea plant with a nodulation score of 2.5 according to the nodulation scale of Corbin *et al*. (1977).

Plate 6: The root system of a pea plant with a nodulation score of 3 according to the nodulation scale of Corbin *et al*. (1977).

Plate 7: The root system of a pea plant with a nodulation score of 4 according to the nodulation scale of Corbin *et al*. (1977).

Plate 8: The root system of a pea plant with a nodulation score of 5 according to the nodulation scale of Corbin *et al*. (1977).

3.3.9 Data Analysis

The data was not tested for normality and heterogeneous variance but was subjected to analysis of variance (ANOVA). Genstat 10.1. Copyright 2007, Lawes Agricultural Trust (Rothamsted Experimental Station) was used for all statistical analyses. Means were separated at the 5% level of significance using the least significance difference (LSD) test for inoculant application, N application, sowing date effects and their interactions.

Chapter 4 Laboratory Materials and Methods

4.1 Recovery of Rhizobia from inoculants

Ten 'Aragon' seeds were surface sterilized in 5 % bleach for five minutes and rinsed three times with sterile water. Seeds were then cultured in water agar (0.15 % agar) in petri dishes covered with lids and placed in plastic bags and were then aseptically geminated in an incubator at 20 °C in the dark for five to seven days. Seedlings were planted in jars filled with autoclaved sterilized vermiculite with each jar containing one seedling. Three seedlings were chosen to be inoculated with a 10 ml mixture of peat inoculant (10 %), a further three were inoculated with a 10 ml mixture of ALOSCA® inoculant (10 %), one seedling was the negative control. The plants were irrigated with a sterile N-free nutrient solution (Sloger, 1969) and placed in a growth chamber at 20 – 22 °C and with 24 hours light. At flowering four nodules, from each treatment, were harvested arbitrarily for Rhizobia culture, cell lysis and DNA extraction, PCR amplification of rhizobial DNA using ERIC primers, F27 and R1494 primers in 16S PCR and DNA analysis.

4.2 *Rhizobium* culture

Pink–red nodules were randomly selected from each plant from the top 50 mm of the tap and lateral roots and were removed by cutting the root approximately 5 mm on either side of the nodule. Nodules were sterilised by immersion in 96 % ethanol for 10 s, followed by 20 % commercial bleach for 2 min and 4 washes in sterile tap water for 20 s each time. The sterile nodules were detached from the root piece and crushed on a sterile Petri plate using a glass rod. A loop full of the bacterial contents of the nodule was streaked onto yeast mannitol agar (YMA: yeast extract 3.663 %, mannitol 36.63 %, K_2HPO_4 1.83 %, $MgSO_4$ 0.73 %, NaCl 0.366 %, $CaCO_3$ 1.83 %, agar 55 %). The YMA plates were incubated at 20 °C for 72 - 168 h. Cultures were sub-cultured from single colonies at least once to ensure purity. Once pure cultures (assessed by visual examination) were obtained they were stored on the YMA plates at 4 °C prior to DNA extraction.

4.3 Cell lysis and DNA extraction

For extraction of bacterial DNA a loop of the culture from a YMA plate was used to inoculate 1 ml of yeast mannitol broth (YMB: Mannitol 84.74 %, Yeast extract 8.47 %, K_2HPO_4 4.24 %, $MgSO_4$ 1.7 %, NaCl 0.85 %). The inoculated YMB was incubated at 28 °C for

48 h at 220 rpm (LABNET 211 DS, Labnet International, USA). Two 1.7 ml tubes containing YMB, but no inoculants, were included to ensure that there was no contamination. The resultant YMB culture was centrifuged at 16,000 × g for 2 min to pellet the cells and the supernatant was discarded. The DNA was extracted from the Rhizobial suspension using the PUREGENE™ (Gentra Systems, USA) DNA extraction kit according to the manufacturer's instructions as follows. Three hundred μl of cell lysis solution was added to the bacterial cells and incubated at 70 °C for 5 min. Following lysis, 1.0 μl RNAse A Solution (Invitrogen) was added, mixed by inverting the tube 25 times and incubated at 37 °C for 15 minutes to degrade RNA. The sample was cooled to room temperature and 100 μl of protein precipitation solution was added, vortexed vigorously and centrifuged at 16,000 × g for 3 minutes. The supernatant was removed to a clean 1.7 ml tube and the DNA was precipitated by the addition of 300 μl of 100 % isopropanol. Samples were inverted gently 50 times and centrifuged at 16,000 × g to pellet the DNA. The DNA was washed with 500 μl of 70 % ethanol and centrifuged again at 16,000 × g for 1 min. The tubes containing the pelleted DNA were allowed to air dry for 15 minutes prior to the addition 30 μl of sterile water. The tubes were left at 4 °C overnight for the DNA to rehydrate. The DNA concentration was quantified by spectrophotometry to determine the average concentration of DNA present in a mixture, as well as the purity. A 2 μl sample droplet was loaded onto an optical pedestal of Nano Drop at wavelengths of 260 and 280 nm. The sample was then measured and diluted with PCR water to a concentration of 50 ng μl^{-1} for optimum performance of the polymerase chain reaction.

4.4 PCR amplification of Rhizobial DNA using ERIC primers

The bacterial DNA was amplified by the polymerase chain reaction (PCR) with primers ERIC 1R (5'-ATGTAAGCTCCTGGGGATTCAC-3') and ERIC2 (5'-AAGTAAGTGACTGGGGTGAGCG-3') (Vachot-Griffin and Thies, 2005). Each 25 μl reaction contained 1X buffer; 200 μM of each dNTPs; 2 μM primer ERIC 1; 2 μM primer ERIC 2; 1.25 U FastStart Taq DNA polymerase (Roche) and 50 ng DNA. The DNA thermal cycle was as follows: initial denaturation at 95 °C for 3 min, followed by 40 cycles of denaturation at 95 °C for 1 minute, annealing at 52 °C for 1 minute and elongation at 72 °C for 1 minute and then a final elongation step at 72 °C for 10 minutes.

4.5 Detection of PCR amplified products

The PCR products were detected by agarose gel electrophoresis. Five μl of PCR product was mixed with 2 μl of loading dye (0.25 % bromophenol blue, 0.25 % xylenecyanol, 40 % sucrose) and separated on a 1 % agarose gel at 10V cm^{-1} for 1 h in 1 x TAE buffer (18 ml distilled H_2O, 0.484 % (w/v) tris base, 1.142 ml glacial acetic acid, 0.0372 % (w/v) EDTA). In the first lane of each gel, 5 μl of 1 kb plus DNA ladder (Invitrogen) was added as a molecular weight marker. The gel was stained for 15 minutes with ethidium bromide (0.5 $\mu g l^{-1}$) and then rinsed in water for 15 minutes. The gel was visualised under UV light and photographed using the Versadoc 3000™ (BioRad) to see variation of the gel pattern.

4.6 DNA sequence

Isolates of the peat inoculant treatment and the ALOSCA® granule treatment from the growth chamber in the laboratory then underwent 16s PCR amplification with two primers F27 and R1494. Each 25 μL PCR reaction contained 2.5 μL of 10 × buffer (FastStart, Roche, USA), 200 μM of each of the dNTPs, 0.25 U *Taq* DNA polymerase (FastStart, Roche, USA) and 10 pmole of each of the primers F27 (5'AGAGTTTGATC(A/C)TGGCTCAG-3') and R1494 (5'CTACGG(T/C)TACCTTGTTACGAC-3') (Weisburg *et al*., 1991; Gomes *et al*., 2001) (Invitrogen). 1 μl of the DNA template diluted down to 20 - 25 ng μl^{-1} was added to each tube. The 16s PCR was run with the thermal cycle: (Veriti™, Applied Biosystems, California, USA): 94 °C for 3 min (denaturation), then 35 cycles of: 94 °C for 30s (denaturation), 55 °C for 30 s (annealing) and 72 °C for 1s (extension), and a final cycle of 72 °C for 7 minutes. After 16s PCR, isolates were sequenced to obtain genus or species level identification. Sequencing was run with primer R1494, at the Lincoln University Sequencing Facility using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, California, USA). Chromas Lite 2.1 (Technelysium Pty Ltd, Australia) was used to view Sequence electropherograms which were then manually trimmed using DNAMAN 4.0 (Lynnon Biosoft, Canada). The DNA sequence results were then compared with database using Basic Local Alignment Search Tool (BLAST) to identify the bacterial strain (Altschul *et al*., 1990).

Chapter 5 Result - Growth, Development and Yield of Peas

Unless otherwise stated there were no significant interactions among sowing date, cultivar or nitrogen source for any of the variables measured.

Unfortunately, severe bird damage decimated the pea population in sub-plots of sowing 2. Therefore accurate data for the LAI, dry matter accumulation and final seed yield could not be collected. However, plant height, leaf number, nodulation assessment, pod characteristic, thousand seed weight and flowering were recorded.

Figure 3: Bird damage at emergence of pea plants sown on 4 November in Canterbury in the 2010/2011 growing season.

5.1 Total dry matter accumulation

Dry matter (DM) accumulation of both pea cultivars increased rapidly during the growing season. The TDM, in the experiment, was influenced by the interaction ($P < 0.05$) between cultivar and sowing date (Figure 4). Dry matter accumulation of 'Aragorn' and 'Ashton' from SD3 (30 November sowing) was greater than from SD1 (15 October sowing) at 45 DAS. However at 60 DAS, there was no difference in the DM of 'Aragorn' and 'Ashton' between SD1 and SD3). At final harvest (75 DAS) there was a difference in the TDM accumulation of Ashton from the 15 October and 30 November sowing. 'Ashton' sown on 30 November accumulated 7.71 t ha^{-1} DM. This was 1.37 t ha^{-1} more than 'Ashton' sown on 15 October. There was no difference in the TDM accumulation of 'Aragorn' between the two sowing dates.

Nitrogen source had no effect on the TDM accumulation of 'Aragorn' and 'Ashton' peas in the experiment (Appendix 1).

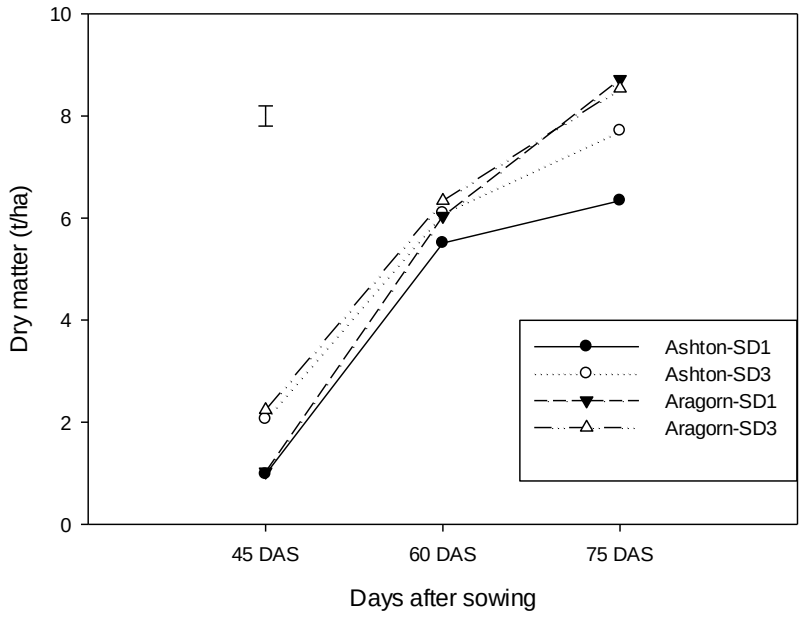

Figure 4: The dry matter accumulation of 'Ashton' and 'Aragorn' peas from different sowings at different sampling dates (Bar is SEM at $P < 0.05$).

5.2 Seed yield

At final harvest the average seed yield was 3.81 t ha^{-1}. Seed yield was influenced ($P < 0.05$) by the interaction of sowing date and cultivar. The highest final seed yield, 4.08 t ha^{-1}

was from 'Aragorn' sown on 15 October 2010. This was 0.7 t ha^{-1} higher than from 'Ashton' (Table 5.1). However from the 30 November sowing there was a similar final seed yield between the two cultivars.

With regard to the different pea cultivars, only 'Ashton' garden pea was influenced by sowing date. The final 'Ashton' seed yield, from the 30 November sowing, was 0.54 t ha^{-1} more than from the 15 October sowing. There was no significant difference in the final seed yield of the field pea between the 15 October and the 30 November sowing (Table 5.1).

Table 5.1: The sowing date x cultivar interaction of seed yield (t ha^{-1}) of peas grown in Canterbury in the 2010/2011 growing season.

Cultivar	**Sowing date**	
	15 Oct	30 Nov
'Ashton'	3.37b	3.91a
'Aragorn'	4.08a	3.90a
Significance	*	
SEM	0.242	
CV (%)	17.9	

5.3 Crop Harvest index

There was no difference in HI among sowing dates, nitrogen source or pea cultivars in the experiment. The mean HI of peas from the 15 October sowing was 0.47 and in the 30 November it was 0.51. The HI of 'Ashton' averagely 0.52 compared with 0.47 for 'Aragorn' (Table 5.2).

5.4 Plant population

Plant population was lower than targeted in both 'Ashton' and 'Aragorn' at each sowing date. The actual plant populations fluctuated from 59 - 80 plants m^{-2} in both the 15 October and 30 November sowing.

Plant population in the experiment was influenced ($P < 0.01$) by the interaction of sowing date and cultivar. There was no difference in the plant population of 'Ashton' between sowing dates at 67 plants m^{-2} from the 15 October sowing and 59 plants m^{-2} from the 30 November sowing. However with 'Aragorn', the number of plants m^{-2} in the 15 October sowing was 65 compared with 80 in the 30 November sowing (Table 5.3). In the 4

November sowing, the plant populations of 'Ashton' and 'Aragorn' were reduced to 32 and 21 plants m^{-2} by bird damage. Nitrogen source had no effect on plant population in the experiment.

Table 5.2: Harvest index of peas grown in Canterbury in the 2010/2011 growing season.

Treatment	Harvest Index
Sowing date	
15 Oct	0.47
30 Nov	0.51
SEM	0.0259
Significance	Ns
Cultivar	
'Ashton'	0.52
'Aragorn'	0.47
SEM	0.0259
Significance	Ns
Nitrogen source	
Control	0.51
50 kg Nitrogen	0.50
Peat	0.48
ALOSCA ®	0.50
SEM	0.0367
Significance	ns
CV%	20.8

Table 5.3: The sowing date x cultivar interaction on plant population (plants m^{-2}) of peas grown in Canterbury in the 2010/2011 growing season.

Cultivar	Sowing date		
	15 Oct	4 Nov	30 Nov
'Ashton'	67.0bc	32.0d	59.0c
'Aragorn'	65.0bc	21.0e	80.0a
SEM		4.53	
Significance		**	
CV%		23.6	

5.5 Number of pods plant^{-1}

The number of pods plant^{-1} was affected by the interaction ($P < 0.01$) of sowing date and cultivar. The different sowing dates had little effect on the number of pods in 'Ashton' but gave a difference in the 'Aragorn' field peas (Table 5.4). The highest number of pods plant^{-1} (10.4) was from 'Aragorn' sown on 4-November and the lowest (5.34) was from 'Ashton' sown on 15-October. Between the two cultivars, 'Aragorn' produced more pods plant^{-1} than 'Ashton' on 15-October and 4-November sowings. The number of pods plant^{-1} of 'Aragorn' and 'Ashton' was similar from the 30-November sowing.

Between cultivars, 'Aragorn' had greater ability to compensate for the low plant population than 'Ashton'. Under the low bird damage population from the 4 November sowing the response of 'Ashton' and 'Aragorn' differed. In 'Ashton', there was no difference in the number of pods plant^{-1} from the 4 November sowing compared with the 15 October and 30 November sowings. In other words, the number of pods plant^{-1} was similar between a population of 32 plants m^{-2} compared with 59 and 67 plants m^{-2}. In contrast, 'Aragorn' compensated for the low plant population by producing only about 30 % more pods plant^{-1} when they were grown at 21 plants m^{-2} compared with 65 and 80 plants m^{-2}.

Table 5.4: The sowing date x cultivar interaction for the number of pods plant^{-1} of peas grown in Canterbury in the 2010/2011 growing season.

Cultivar	**Sowing date**		
	15-Oct	4-Nov	30-Nov
'Ashton'	5.34c	6.22bc	6.15bc
'Aragorn'	7.15b	10.4a	6.90b
Significance		**	
SEM		0.734	
CV (%)		29.5	

5.6 Pod length

In the field experiment, there was an interaction between sowing date and cultivar ($P < 0.05$) for the length of garden and field pea pods. The pods of 'Ashton' were always longer than the pods of 'Aragorn'. For 'Ashton' November sowing produced pods of 75.9 mm and 74.6 mm compared with 67.6 mm from the 15 October sowing. In 'Aragorn', the

mean pod length of the 4 November sowing was 68 mm compared with 63 mm from the 30 November sowing and 58.8 mm from the 15 October sowing (Table 5.5).

Table 5.5: The sowing date x cultivar interaction on pod length (mm) of peas grown in Canterbury in the 2010/2011 growing season

Cultivar	**Sowing date**		
	15 Oct	4 Nov	30 Nov
'Ashton'	67.6b	74.6a	75.9a
'Aragorn'	58.8d	68.0b	63.0c
Significance		**	
SEM		1.461	
CV (%)		6.1	

5.7 Number of seeds pod^{-1}

The number of seeds pod^{-1} was influenced by the interaction ($P < 0.01$) between sowing date and cultivar. 'Ashton' produced more seeds pod^{-1} than 'Aragorn' at all sowings (Table 5.6). The number of seeds pod^{-1} of plants sown in November was greater than in plants sown in October. 'Ashton' had an average of 6.23 seeds pods^{-1} from the 4 November sowing and 6.14 seeds pod^{-1} from the 30 November sowing compared with 5.64 in the October sowing. In 'Aragorn' pea, the figures were highest at 5.30 seeds pod^{-1} from 4 November and lowest at 3.80 seeds pod^{-1} from the 15 October sowing.

Table 5.6: The sowing date x cultivar interaction on number of seeds pod^{-1} of peas grown in Canterbury in the 2010/2011 growing season.

Cultivar	**Sowing date**		
	15 Oct	4 Nov	30 Nov
'Ashton'	5.64b	6.23a	6.14a
'Aragorn'	3.80d	5.30b	4.21c
Significance		**	
SEM		0.1743	
CV (%)		9.4	

5.8 Thousand seed weight

Sowing date had no effect on the 1,000 seed weight of 'Ashton' pea but gave differences ($P < 0.01$) in 'Aragorn'. In 'Aragorn', the 4 November sowing had the highest

thousand seed weight at 225 g, followed by the 15 October sowing (209 g) and the 30 November sowing (169 g). 'Aragorn' had a higher thousand seed weight than 'Ashton' in the two early sowings. In contrast, 'Ashton' had a higher thousand seed weight than 'Aragorn' at the late November sowing (Table 5.7). Nitrogen source had no effect on the 1000 seed weight in the experiment.

Table 5.7: The sowing date x cultivar interaction for the thousand seed weight (g) of peas grown in Canterbury in the 2010/2011 growing season.

Cultivar	**Sowing date**		
	15 Oct	4 Nov	30 Nov
'Ashton'	181c	191c	187c
'Aragorn'	209b	225a	169d
Significance		**	
SEM		6.58	
CV (%)		9.6	

5.9 Green area index

The green area index (GAI) increased rapidly during vegetative growth. Nitrogen source had no effect on the GAI in the experiment but sowing date ($P < 0.01$) did. During the growing season, the GAI from the 30 November sowing was higher than in the 15 October sowing at all samplings (Figure 5). At the 40 DAS sampling, the GAI of the 30 November sowing was 1.55 which was nearly three times that of 15 October sowing at 0.57. At 50 and 60 DAS the gap between two sowing dates was smaller. The average GAI of the 15 October sowing was 2.02 and 2.54 at the 50 and 60 DAS samplings respectively. The GAI for these two sampling dates for the 30 November sowing was 2.58 and 3.12, respectively.

There was no significant difference ($P = 0.5$) in the GAI between sowing dates for both 'Ashton' and 'Aragorn'. At flowering the GAI of 'Ashton' from the 15 October and 30 November sowing was 2.38 and 2.92. The GAI of 'Aragorn' from the 15 October and 30 November sowing was 2.7 and 3.32 (Figure 6).

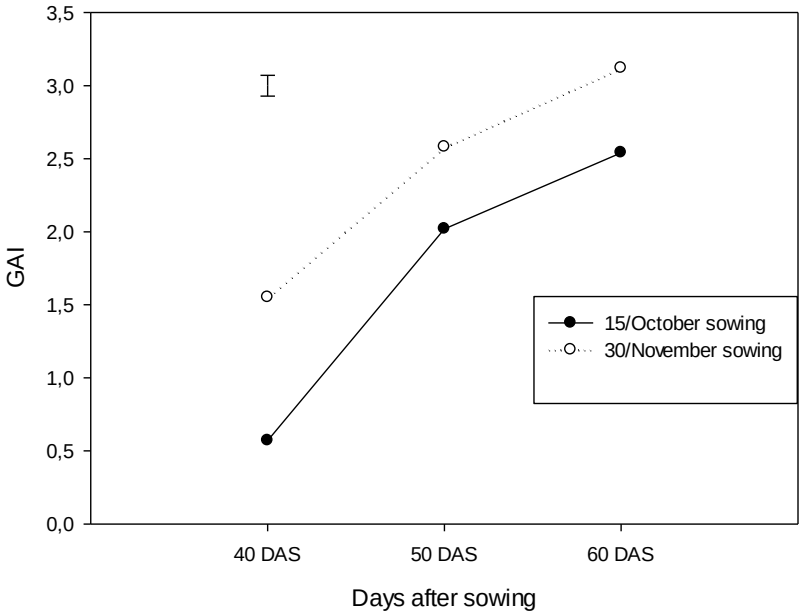

Figure 5: Green area index of different sowing dates of peas grown in Canterbury in the 2010/2011 growing season (Bar is SEM at P < 0.01).

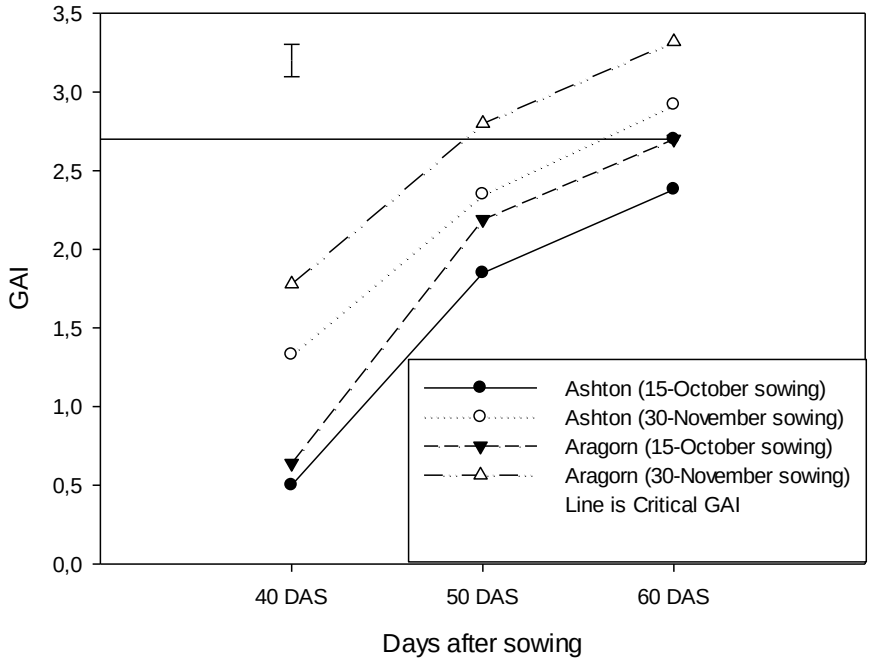

Figure 6: Green area index of 'Ashton' and 'Aragorn' peas from different sowing dates in Canterbury, New Zealand in the 2010/2011 growing season (Bar is SEM at P = 0.05).

5.10 Plant height

In the experiment, plant height was influenced by the interaction of sowing date and cultivar. During the growing season, plants from the 30 November sowing were taller ($P < 0.01$) than both 15 October and 4 November sowings. At 65 the DAS sampling, 'Aragorn' and 'Ashton' in the 30 November sowing had a final plant height of 623 and 531 mm, respectively. The final plant heights of 'Aragorn' and 'Ashton' from the 15 October sowing were 518 and 402 mm. The figures for the bird damaged 4 November sowing were 441 and 384 mm (Figure 7).

There was no difference in plant height between 'Aragorn' and 'Ashton' peas during their early growth. However, later, at the 55 and 65 DAS samplings 'Aragorn' had a longer stem than the 'Ashton' in all sowings.

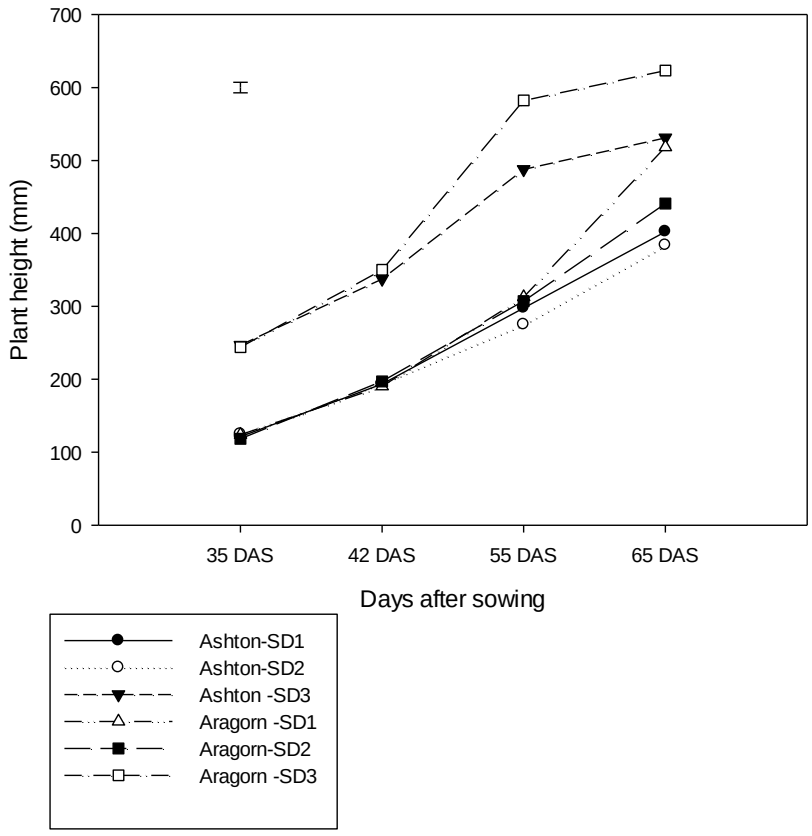

Figure 7: Plant height of 'Ashton' and 'Aragorn' at different sowing dates in Canterbury, New Zealand in the 2010/2011 growing season (Bar is SEM at $P < 0.01$).

5.11 Phyllochron

The results showed that at base temperature of 4.5°C the phyllochron in this field experiment was only affected ($P < 0.01$) by cultivar (Table 5.8). The fastest leaf appearance rate was 16.2 °C d for 'Aragorn' compared with 27.9 °C d for 'Ashton'.

Table 5.8: The phyllochron of peas grown in Canterbury in the 2010/2011 growing season.

	Phyllochron
Sowing date	
15 Oct	21.2
4 Nov	23.0
30 Nov	22.0
SEM	2.275
Significance	ns
Cultivar	
'Ashton'	27.9a
'Aragorn'	16.2b
SEM	1.858
Significance	**
Nitrogen source	
Control	19.8
50 kg nitrogen	23.6
Peat	22.8
ALOSCA®	22.0
SEM	2.627
Significance	ns
CV%	21.2

5.12 Days to flowering and thermal time units from emergence to flowering

The results from the field experiment showed that there was a difference ($P < 0.01$) in the time from emergence to flowering among sowing dates. Sowing date 1 required 41.2 days to reach flowering, sowing dates 2 and 3 required 36.9 days and 34.4 days to reach flowering, respectively (Table 5.9).

The mean number of days to flowering was 37.0 for 'Ashton' and 38.1 for 'Aragorn' ($P < 0.01$). Despite these differences the thermal time requirement from emergence to flowering was 436 °C d for 'Ashton' and 446 °C d for 'Aragorn' ($P < 0.05$) and was consistent across sowing dates.

Table 5.9: Days to flowering and thermal time from emergence to flowering of two pea cultivars sown in Canterbury during the 2010/11 growing season.

	Days to Flowering	Thermal time from emergence to flowering (tb = 4.5°C)
Cultivar		
'Ashton'	37.0b	436b
'Aragorn'	38.1a	446a
Significance	**	*
SEM	0.327	4.33
Sowing date		
15 Oct	41.2a	441
4 Nov	36.9b	438
30 Nov	34.4c	444
Significance	**	ns
SEM	0.40	5.31
CV%	4.3	4.8

Chapter 6 Result – Pea Nodulation

6.1 Recovery of *Rhizobium* from commercial inoculants.

Genotypic characterization was performed on nine bacteria extracted and isolated from the nodules of four pea plants inoculated with peat inoculant and ALOSCA® granules grown in a growth chamber and shown in Plate 9.

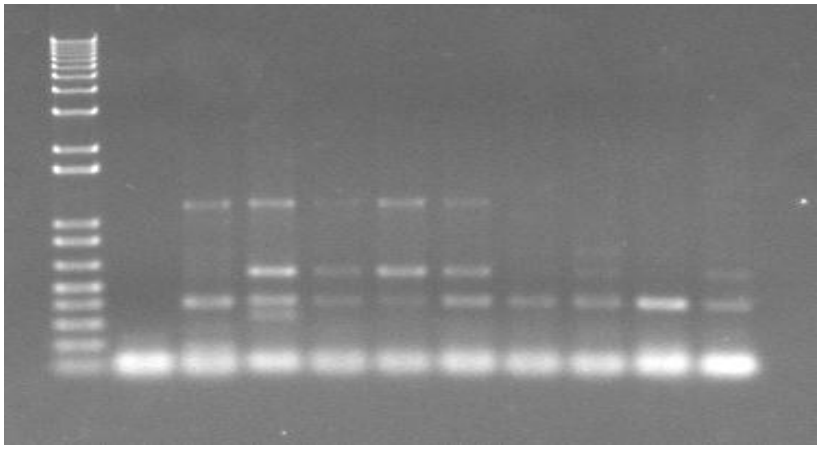

Plate 9: Agarose gel showing representatives of Unique ERIC-PCR fingerprints obtained using DNA extracted from the bacteria recovered from pea plant nodules inoculated with commercial inoculants and grown in a growth chamber. Lane 1 contains the 1Kb Plus DNA Ladder™ (Invitrogen).

The results, after comparison of the DNA sequence of these genotypes with the gene bank in BLAST showed that they were all *Rhizobium leguminosarum* spp. This shows that there were *Rhizobium leguminosarum* spp in both of the commercial inoculants and they had the ability to induce nodulation of peas.

6.2 Nodulation of pea in the field under different nitrogen sources.

All assessed plants had nodules and the nodules tended to be located below 50 mm on the root system. The nodulation score of pea plants in the field experiment was affected by an interaction ($P < 0.01$) between sowing date and nitrogen source (Table 6.1). ALOSCA® granules resulted in a higher nodulation score than most of the other treatments at all sowing dates except for peat inoculant in the 30 November sowing. The ALOSCA® granules, applied in November, gave a higher nodulation score than the October sowing. The mean nodulation score of plants inoculated with ALOSCA® granules was 2.40 and 2.48 from the 4

November and 30 November sowings compared with 2.26 for the 15 October sowing. Plants treated with peat inoculant had higher nodulation scores than the control treatments at all sowings. In the 15 October sowing the nodulation scores were 1.96 from peat inoculant and 1.67 for the control. In the 30 November sowing, nodulation score of the peat treatment and the control increased to 2.43 and 2.26, respectively. The nodulation score of plants which received 50 kg N ha^{-1} was higher than in control plants in the 15 October and 4 November sowings but not from the 30 November sowing.

Table 6.1: The sowing date x cultivar interaction on nodulation of peas grown in Canterbury in the 2010/2011 growing season.

Treatment	**Control**	**50 kg N**	**Peat inoculant**	**ALOSCA® granule**
15 Oct	1.67	1.83	1.96	2.26
4 Nov	1.60	1.87	2.13	2.40
30 Nov	2.26	2.30	2.43	2.48
SEM			0.066	
Significance			**	
CV%			6.5	

Chapter 7 General Discussion

7.1 Total dry matter accumulation

In the field experiment, TDM accumulation of 'Ashton' was influenced by the interaction of sowing date by cultivar. At final harvest, 'Ashton' sown on 30 November produced 1.37 t DM ha^{-1} more than the 15 October sowing. There was no difference in TDM accumulation of 'Aragorn' between the 15 October and 30 November sowings and they both accumulated over 8.5 t DM ha^{-1} (Figure 4). The difference in final TDM accumulation of 'Ashton' between the 15 October and 30 November sowings was probably due to the difference in GAI. 'Aragorn' and 'Ashton' are determinate cultivars so GAI, at flowering, was at a maximum because there was no further leaf appearance afterwards. The GAI at flowering, of 'Ashton' from the 15 October sowing, was lower than the critical GAI for peas in New Zealand which is reportedly 2.7 (Munakamwe, 2008). The GAI of 'Ashton' from the 15 October sowing was 2.38 compared with 2.92 from the 30 November sowing (Figure 6). The GAI, at flowering, was below the critical GAI for peas in New Zealand for 'Ashton' from the 15 October sowing. This meant that less radiation was intercepted by the canopy and the plants were less competitive to weeds. Because, in the experiment, as weed control was applied before the Sowing 1 there were only a few weeds in the Sowing 1 experimental plots. However, by the end of the experiment weeds were developing faster and were more common in the Sowing 3 experimental plots and surrounding areas. This resulted in a lower DM accumulation compared with the 30 November sowing. The GAI, at flowering, of 'Aragorn' was 2.70 from the 15 October and 3.32 from the 30 November sowings and they both met the critical LAI for peas in New Zealand. This explains why there was no significant difference in TDM accumulation of 'Aragorn' between sowing dates.

The relationship between TDM accumulation of peas and the proportion of solar radiation intercepted by the canopy foliage was confirmed by Heath and Hebblethwaite, (1987). Solar radiation intercepted depends on the GAI because leaves are the most important photosynthetic organs. Crop growth rate per unit green area increases as GAI increases until most of the incident light is intercepted (Brown *et al*., 1968). As shown in previous field experiments, a GAI of 2.7 is needed in New Zealand (Munakamwe, 2008), for the interception of 95 % of the photosynthetically active radiation. Moreover, competition

for light is an important determinant of pea crop's ability to compete with weeds. Plants with a higher GAI accumulated more intercepted radiation and competed more efficiently with weeds than plants with a lower GAI. Lantinga *et al*. (1999) concluded that the competitive success of clover over grass for light absorption was due to its greater contribution to total GAI. Kosgey (1994), obtained a similar result in a chickpea experiment and found that DM accumulation, in the field, increased as GAI increased.

In this experiment there was no difference in the plant population of 'Ashton' between the 15 October and 30 November sowings. However, differences between the two sowing dates were found in 'Aragorn'. The plant population of 'Aragorn' in the 30 November sowing was 80 plants m^{-2} which was 15 plants m^{-2} more than in the 15 October sowing (Table 5.3). This could contribute to a 1.21 t ha^{-1} higher DM from the 30 November sowing compared with the 15 October sowing at the 45 DAS sampling (Figure 4). It is possible that 'Aragorn' canopies closed faster from the higher populations and therefore intercepted more radiation than the lower population. McKenzie and Hill (1991) found that when crops have adequate soil moisture and fertility, high plant populations closed their canopies earlier, intercepted more radiation and this resulted in an early rapid growth rate. In this experiment, soil moisture and fertility were not limiting factors and therefore with a higher population, 'Aragorn' from the 30 November sowing could accumulate more DM in early vegetative growth than from the 15 October sowing. However, there was no difference in final TDM accumulation of 'Aragorn' from the 15 October and the 30 November sowings. At flowering, 'Aragorn' from both sowing dates achieved a GAI above the critical GAI for peas in New Zealand. Thus their canopies intercepted sufficient light for the crop growth. This is consistent with the report of Ambrose and Hedley (1984) who found that plant population often has no effect on the biological yield of peas. Munakamwe (2008) found that there was no difference in the TDM of 'Aragorn', at harvest, among the population of 50, 100 and 400 plants m^{-2} because 'Aragorn' had the ability to compensate for low plant population by producing more branches. Besides, the 30 November sowing was 1 month later than the recommended sowing time for 'Aragorn' and this resulted in a decrease in 'Aragorn' seed weight. Low seed weight is another reason for there being no difference in the TDM accumulation of 'Aragorn' between the 15 October and 30 November sowings although the 30 November sowing had a higher plant population. In contrast, the population of 'Ashton'

was similar between the two sowing dates. However, TDM accumulation from the 30 November sowing was higher than from the 15 October sowing in both the early and final samplings. The lower TDM accumulation of 'Ashton' from the 15 October sowing compared to the 30 November sowing was because it did not intercept sufficient light for growth. To achieve high yields, crops should produce enough GAI as fast as possible to intercept most of the incident light (Ayaz, 2001). At flowering the GAI of 'Ashton' from the 15 October sowing was 2.38 (Figure 6) which is lower than the critical GAI for peas in New Zealand. This means that throughout the growing season, 'Ashton' peas sown on 15 October did not produce enough GAI to intercept sufficient solar radiation and utilize it for TDM production.

Nitrogen application and *Rhizobium* inoculation had no effect on the final DM accumulation of peas in this experiment. A satisfactory soil nitrogen content was attributed to the absence of a response of the peas to nitrogen application and *Rhizobium* inoculation on TDM. The soil nitrogen test result, at flowering, revealed that the soil nitrogen content was still equivalent to 43 kg N (32.5 kg nitrate) ha^{-1} in the 15 October plots and 58 kg N (54 kg nitrate) ha^{-1} in the 30 November plots. This was after the majority of the vegetative DM had been grown and suggests that there was adequate soil N at the beginning of the experiment to grow the crop. This result is supported by previous experiments in New Zealand that showed the application of N fertilizer at up to 60 kg N ha^{-1} had no influence on shoot DM accumulation (Gunawardena *et al*., 1997).

7.2 Pea seed yield

The overall mean seed yield of the field trial was 3.81 t ha^{-1}. This was higher than the average pea seed yield in New Zealand and the average global pea seed yield. The average global pea seed yield was 1.62 t ha^{-1} and the average pea seed yield in New Zealand was 3.09 t ha^{-1} (FAOSTAT, 2010). However, this did not provide an economic benefit as Freeman (1987) confirmed that pea is required to have a final seed yield over 4 t ha^{-1} to be an economically viable crop in New Zealand (Freeman, 1987). The unsatisfactory final seed yield in the trial was attributed to the lower than target population. In New Zealand, a plant population of 80 - 100 plants m^{-2} is optimal for field peas and the target may be higher for garden peas (PIDG, 2008). However, in this experiment, although the target population was 100 plants m^{-2}, the actual populations were always lower than the optimal population (Table 5.3). The reason was the difference between the emergence test in the growth chamber and

the emergence rate in the field. The emergence test showed that the emergence of 'Aragorn' and 'Ashton' was 98 % and the sowing rate was calculated based on this figure. However, in the field, the practical emergence rate was 60 – 80 % and this contributed to the low population of this experiment. To achieve a population of at least 100 plants m^{-2} an emergence rate of 60 - 80 % is recommended for use in calculating the sowing rate.

For the 4 November sowing, emergence occurred in mid-November and there was little other food source in the field for pigeons. Pea seedlings are a good protein source and they were eaten by pigeons. This resulted in an actual plant population of just 20 - 30 % of expected.

In this experiment, only 'Aragorn', sown on 15 October, gave a final seed yield of over 4 t ha^{-1}. However, there was no significant difference in the final seed yield of 'Aragorn' between the 15 October and 30 November sowing as there was only a 0.20 t ha^{-1} difference from each other (Table 5.1). However, there was a difference in the final seed yield of 'Ashton' where the 30 November sowing produced nearly 0.6 t ha^{-1} more than the 15 October sowing. Seed yield was shown to be correlated with DM accumulation (Dai *et al*., 2005). In this experiment 'Ashton', sown on 30 November, accumulated 1.37 t DM ha^{-1} more than the 15 October sowing (Figure 4) and there was no difference in the HI among sowing dates (Table 5.2). This gave a greater seed yield of 'Ashton' from the 30 November sowing compared with the 15 October sowing. There was also no difference in the final TDM accumulation and HI of 'Aragorn' between the two sowing dates. As a result there was no difference in the final seed yield.

In peas, leaves, stipules and tendrils are important photosynthetic organs. To achieve high yield, it is required to quickly produce sufficient GAI to intercept most of the incident light (Ayaz, 2001). The aim is to maintain a high level of interception and partition as much assimilate as possible to the reproductive organs (Gardner *et al*., 1985). Kruger (1977) found that when a pea population had a LAI of 5.3 or higher, over 95 % of incoming solar radiation was intercepted by the crop canopy. In another experiment in New Zealand, irrespective of sowing date, a LAI value of 2.7 was sufficient to intercept 95 % of the photosynthetically active radiation (Munakamwe, 2008). In the current experiment the GAI, at flowering of 'Ashton', in the 15 October sowing was 2.38 (Figure 6) which was lower than

the critical GAI (Munakamwe, 2008). The GAI at flowering of 'Ashton' in the 30 November sowing was 2.92 which is higher than the critical GAI for peas in New Zealand. Both 'Ashton' and 'Aragorn' are determinate cultivars therefore their GAI, at flowering, is at a maximum. The lower than critical GAI of 'Ashton' from the early sowing gave less light interception for the duration of pod filling. The GAI, at flowering, of 'Aragorn' in the 15 October sowing and the 30 November sowing was 2.7 and 3.3 respectively. Both these values were sufficient to maximise light interception.

In this experiment, 'Aragorn' showed a greater ability than 'Ashton' to compensate for the low plant population in the 4 November sowing. At the low population, due to bird damage, 'Aragorn' produced around 3 pods plant^{-1} more than the 15 October and 30 November sowings (Table 5.4). For 'Aragorn' to maintain the same seed yield as obtained, at the higher population, it would need about 30 plants m^{-2}. 'Ashton' had less ability to compensate for the low plant population and would need 55 plants m^{-2} to produce the same seed yield as from the 30 November sowing.

Neither *Rhizobium* inoculation nor N application affected seed yield (Appendix 2). This result is similar to that of Begum *et al*. (2001) where *Rhizobium* inoculation increased pea nodulation and pod number plant^{-1} but had no impact on the final seed yield (Askin *et al*., 1985). In New Zealand, *Rhizobium* inoculation had no effect on nitrogen fixation, growth and seed yield of peas (Kelstrup *et al*., 1996). Ram and Sanoria, (1979) reported that pea yield was not correlated with the number of nodules plant^{-1}. It is noticeable that the majority of *Rhizobium leguminosarum* bv. *viceae* have more effect on nitrogen accumulation in the plant than on shoot mass (Fesenko *et al*., 1995). Where *Rhizobium* has a positive effect on both N accumulation and seed yield, the mean value of the increase is higher for N accumulation (Fesenko *et al*., 1995). This conclusion is confirmed, again, with the data of Skot, (1983). Soil nitrogen is important in the determination of the response of pea to *Rhizobium* inoculation or nitrogen fertilizer application. Symbiotic nitrogen fixation may begin to be limited when nitrate content of the ploughed layer is greater than 14 kg ha^{-1}. Instantaneous % Ndfa decreased linearly from 100 to 0 % as the soil nitrate content increased from 0 to 34 kg ha^{-1} (Voisin *et al*., 2002). The satisfactory soil nitrogen level of 32.5 to 54 kg nitrate ha^{-1}, in the experimental site soil, at flowering, and the small difference in

the nodulation score, among treatments, in this field experiment (Table 6.1) could explain why there was no response of pea seed yield to *Rhizobium* inoculation and N application.

7.3. Crop Harvest index

Difference in crop harvest index (CHI) in the experiment may have been expected because HI of peas is variable and differs among cultivars (Ambrose and Hedley, 1984; Srivastava and Asthana, 1994; Ayaz *et al.*, 2004). However, there was no difference in the CHI among cultivars or sowing dates. The CHI in this experiment was 0.52 for 'Ashton' and 0.47 for 'Aragorn' (Table 5.2). These values were relatively high when compared with CHI of peas in previous experiment by Askin, (1983) and Moot, (1993). The CHI is influenced by many factor such as cultural practice, the partition of assimilates to seed, growth duration and genotype (Ayaz *et al.*, 2004). 'Aragorn' and 'Ashton' are both semi-leafless genotypes, the received the same cultural practices during the experiment and their growth duration was similar and these help explain why no difference in CHI may have been found. Although there was no statistical difference in CHI between the two cultivars the CHI of 'Aragorn' at 0.47 compared with 0.52 of 'Ashton' was probably due to more assimilates being used to support the greater height in the vegetative growth of 'Aragorn' (Figure 7).

7.4 Number of pods plant^{-1}

The number of pods plant^{-1} varies among cultivars. Old cultivars bear a single pod node^{-1} therefore there are not many pods plant^{-1} whereas newer, advanced cultivars may bear up to five pods node^{-1} giving a dramatic increase in the number of pods plant^{-1} (Knott, 1987). In this field experiment all treatments produced 5 to 10 pods plant^{-1}. Pod number was influenced by the interaction between sowing date and cultivar (Table 5.4). 'Aragorn' produced more pods plant^{-1} than 'Ashton'. This is probably genetically controlled and is therefore a heritable trait (Knott, 1987; White, 1987). There was no difference in pod number plant^{-1} between Sowings 1 and 3 in both the 'Ashton' and 'Aragorn'. 'Aragorn', but not 'Ashton', compensated for the low plant population due to bird damage in the 4 November sowing. 'Aragorn' in Sowing 2 had nearly 3 pods plant^{-1} more than in the other two sowings. The greater number pod plant^{-1} in sowing 2 was probably compensation for the low plant population that decreased interplant competition for light. This confirmed the report of El-Habbasha *et al*. (1996) who found that increased plant density decreased pod

yield plant^{-1} and pods plant^{-1}. Nleya and Rickertsen, (2011) reported that pea plants compensated for low plant populations by producing more pods plant^{-1} and more seeds pod^{-1}. Other experiments in Canterbury, New Zealand have shown a similar trend with a reduction in pods plant^{-1} and seeds pods^{-1} when plants were grown at a higher population (Falloon and White, 1978; McKenzie *et al*., 1986; McKenzie and Hill, 1995).

In addition, it was observed, in this experiment, 'Aragorn' showed excellent lodging resistance and it facilitated plants standing up to intercept maximum radiation for growth and development at a low plant population. In contrast, 'Ashton' has lower lodging resistance and under a low population, they did not intertwine to support each other to stand up and prevent their collapse to intercept maximum radiation.

In the field, the highest final pea yield was normally achieved with population around 100 plants m^{-2} as increased plant density prolongs the reproductive phase (Sawicki *et al*., 2000). The 60 - 80 plants m^{-2} achieved in the current experiment appears to have been below that required to maximize yield. Further, 'Aragorn' had some ability to compensate for a low plant population. These results suggest that for semi-leafless, determinate cultivars establishing an adequate plant population is critical to obtain an economic yield. Early sown 'Ashton' should probably be sown at higher rates because it has less ability to compensate for a low plant population than 'Aragorn'.

7.4 Number of seeds pod^{-1}

Under a wide range of conditions, seed number pod^{-1} has been considered as the most variable yield component in grain crops and is usually positively correlated with final seed yield (Poggio *et al*., 2005). In this experiment there was an interaction of sowing date by cultivar which affected the number of seeds pod^{-1}. 'Ashton' had more seeds pod^{-1} than 'Aragorn' at the different sowing dates (Table 5.6). As for pod plant^{-1} this is a genetically controlled heritable characteristic (Knott, 1987; White, 1987). Experimental data showed that 'Ashton' had longer pods than 'Aragorn' (Table 5.5) and therefore they can contain more seeds in each pod. In addition, each pea cultivar responded differently to the variation in experimental conditions in the process of seed formation.

In the experiment 'Aragorn' increased seed pod^{-1} to compensate for a low plant population. 'Aragorn', in Sowing 2, had more seeds pod^{-1} than in both Sowings 1 and 3.

'Aragorn' in Sowing 3 had more seeds pod^{-1} than in Sowing 1. 'Ashton' in Sowing 2 had more seeds pod^{-1} than in Sowing 1 but had the same number of seed pod^{-1} as Sowing 3. The result confirmed the report of McKenzie *et al*. (1986) who seed pod^{-1} number decreased with increased plant population and was increased in a later sowing. The low plant population may have decreased the frequency of seed abortion and therefore given larger pods which contained more seeds pod^{-1} (Jeuffroy and Chabanet, 1994; El-Habbasha *et al*., 1996). In addition, the number of pods and seeds in peas is positively related to radiation interception during seed set (Poggio *et al*., 2005). Under a low plant population, each plant can intercept more radiation and increase its photosynthesis to partition more assimilates to reproductive organs, during the critical period of seed set, and as a result increase seed pod^{-1} (Bruening and Egli, 1999).

In the experiment, seed pod^{-1} was positively correlated with pod length. 'Ashton' in the 4 and 30 November sowings had longer pods than in the 15 October sowing. Consequently, the number of seed pod^{-1} from the 4 and 30 November sowings was similar and greater than in the 15 October sowing. The pod length of 'Aragorn' was greatest in the 4 November sowing and was followed by the 30 November and 15 October sowings respectively. The seed pod^{-1} was highest in the 4 November sowing, followed by 30 the November and 15 October sowings.

7.5 Thousand seed weight

The thousand seed weight is also a heritable genetic characteristic and depends on management and growing conditions (Falloon and White, 1978; Wilson, 1987; Castillo *et al*., 1994; Poggio *et al*., 2005). Field peas, in Sowing 2, which had the lowest populations, also had the highest thousand seed weight at 225 g. Sowing 1 with a population of 65 plant m^{-2} had a thousand seed weight of 209.1 g. Sowing 3 plants with a population of 80 plant m^{-2} had the lowest thousand seed weight at 169 g (Table 5.7). This was because, at a low plant population, there was less competitive pressure for seed filling per unit area. Moot, (1993) also reported a decrease in the mean seed weight of field peas with increased plant population. The mean seed weight of 'Ashton' was similar among the different sowing dates. This once again showed the greater ability of 'Aragorn' to compensate for a low plant population. 'Aragorn' at a low plant population had more pods and seed and had heavier seed. In contrast, these variables in 'Ashton' were similar from the population of 30

compared with 65 plant m^{-2}. The lower ability to remain erect and intercept as much radiation in the low plant population of 'Ashton', compared to 'Aragorn', explained why there was no significant difference in seed weight among sowing dates.

The heavier seed of 'Aragorn', in Sowing 1, compared with Sowing 3 was attributed to the adjustment between seed size and other yield components. There was compensation between seed number and seed weight in the large seeded cultivar but not in the small seeded cultivar. This was probably due to source-limitation as assimilate production may be insufficient when a high number of large seeds are set (Poggio *et al*., 2005). Field peas, in Sowing 3, had more seed pod^{-1} than in Sowing 1. To compensate Sowing 1 had a higher mean seed weight. In addition, the typical sowing window for 'Aragorn' is strictly suggested as from September to the end of October (PIDG, 2008). In this experiment, the last sowing was on 30 November which meant 'Aragorn' was sown 1 month later than recommended. The one month delay in sowing may have contributed to the decrease in mean seed weight of 'Aragorn' in the 30 November sowing compared with the 15 October sowing. As the crop was sown late, and outside the typical sowing window, the growing conditions were not optimal for growth and development. In addition, the crops developed faster and this meant that the reproductive stage was shortened, and less radiation was intercepted. As a result less photosynthate was transported to seed. This result is similar to that obtained of Gubbels, (1977) who found a significant decline in the 1,000 seed weight when sowing was delayed. Although 'Aragorn', from the 30 November sowing had a lower mean seed weight than in the 15 October sowing there was no significant difference in the final seed yield. This was because the plant population in the 30 November sowing was higher and it was compensated for by a lower mean seed weight. 'Ashton' had smaller seed than 'Aragorn' so probably there was no compensation between seed number and seed weight. Further, in the experiment, 'Ashton' was sown at the recommended sowing time and this explains the no difference in seed weight among the three sowing dates.

7.6 Green area index

Although there was no statistical difference in GAI at flowing of the two different cultivar the GAI of 2.38 of 'Ashton' from the 15 October sowing was consider insufficient for peas in New Zealand. In this experiment, 'Aragorn' had a faster rate of leaf appearance than 'Ashton'. 'Aragorn' required 16.2 $^{\circ}$C d and 'Ashton' needed 27.9 $^{\circ}$C d for one leaf to appear

(Table 5.8). Each cultivar required a certain amount of thermal time for a leaf to appear therefore the canopy development of 'Ashton' and 'Aragorn' depended on the temperature during their growing season. The mean temperature, during the growing season, of plants from the 30 November sowing was higher than in the 15 October sowing and this produced a GAI, below the critical value in 'Ashton' sown on 15 October (Figure 6) and a higher mean GAI of peas from the 30 November sowing compared to the 15 October sowing (Figure 5).

7.7 Days to flowering and thermal time units from emergence to flowering

In this experiment 'Ashton' required 37 days (436 $^{\circ}$C d) and 'Aragorn' required 38 days (446 $^{\circ}$C d) from emergence to flowering (Table 5.9). The difference in the number of days to flowering between the garden pea and the field pea was small and is genetically control. Different pea cultivars require a different time to reach flowering and maturity (Agarwal *et al*., 2006).

Temperature is an important factor in determining the rate of pea development. Under the effect of temperature a cultivar will not always need the same amount of calendar time but it will always need the same amount of thermal time to reach a certain developmental stage (Olivier and Annandale, 1998). In the field experiment, a delay in the sowing date meant plants were exposed to warmer temperatures as the season changed from spring to summer. This meant that Sowing 3 accumulated higher thermal unit days^{-1} than Sowings 2 and 1, respectively. That is why plants from Sowing 3 required the shortest number of days to reach flowering but needed the same total thermal units.

7.9 Nodulation of pea

7.9.1 Inoculation response

In this experiment *Rhizobium* inoculation resulted in statically higher nodulation in both garden and field peas. However, this difference was considered statistical rather than physiological. The response is considered weak, and insufficient, as indicated by the low of nodulation scores across all treatments in the experiment (Table 6.1). A crop is considered to have satisfactory nodulation when it has a mean nodule score ≥ 3 (Fettell *et al*., 1997). This is higher than the scores of 1.60 to 2.46 attained in this experiment. Therefore, the small difference in nodulation scores, probably did not result in a positive response in final TDM accumulation and seed yield. The data from this field experiment confirmed the result,

obtained previously from a field experiment by Kelstrup *et al*. (1996) who found that *Rhizobium* inoculation had no effect on N fixation of peas in New Zealand. He also found that the *Rhizobium*, isolated from plant roots, was mainly from the soil rather than the applied inoculant.

Between the two inoculant types, ALOSCA® granules gave a higher nodulation score than the peat inoculant. Inoculation method also affected nodule distribution on the root system. The peat inoculant resulted in the most nodules distributed on the tap root and from 0 - 5 cm below the stem base (Plate 10). In contrast, the granular inoculant applied into the soil resulted in more nodules in lower positions from 5 - 15 cm below the stem base (Plate 11). The nodule distribution pattern of the peat inoculant and the ALOSCA® granule, in this experiment, support the finding of Hardarson *et al*. (1989). Results from field peas, chickpeas and lucerne experiments have also shown that granular inoculant application to soil gave higher nodule numbers and nodule mass than peat inoculant (Brockwell *et al*., 1980; Clayton *et al*., 2004b). However, Khumalo, (2012) and Wigley, (2012) have both recently shown that ALOSCA® granules, peat inoculant and coated seed all resulted in poor delivery of *Rhizobium* to lucerne nodules in Canterbury. A recent decision, by one commercial company, not to inoculate white clover seed in New Zealand also suggests the indigenous population may now be sufficient for clover growth. For peas, the current results showed no yield benefit to the use of an inoculant and thus *Rhizobium* inoculation is not certain of marking positive effect in New Zealand.

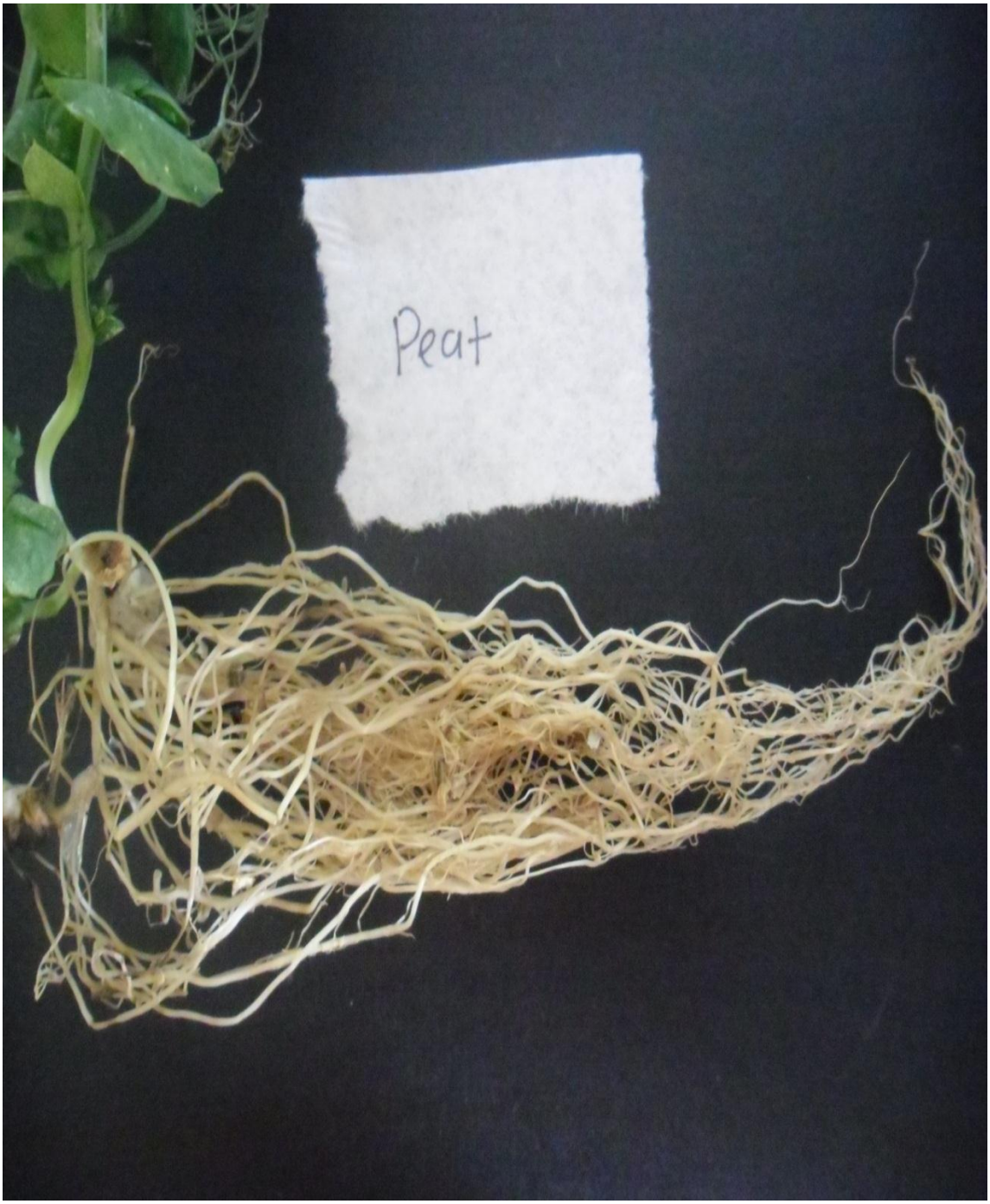

Plate 10: Nodulation parttern of peas under peat inoculant treatment

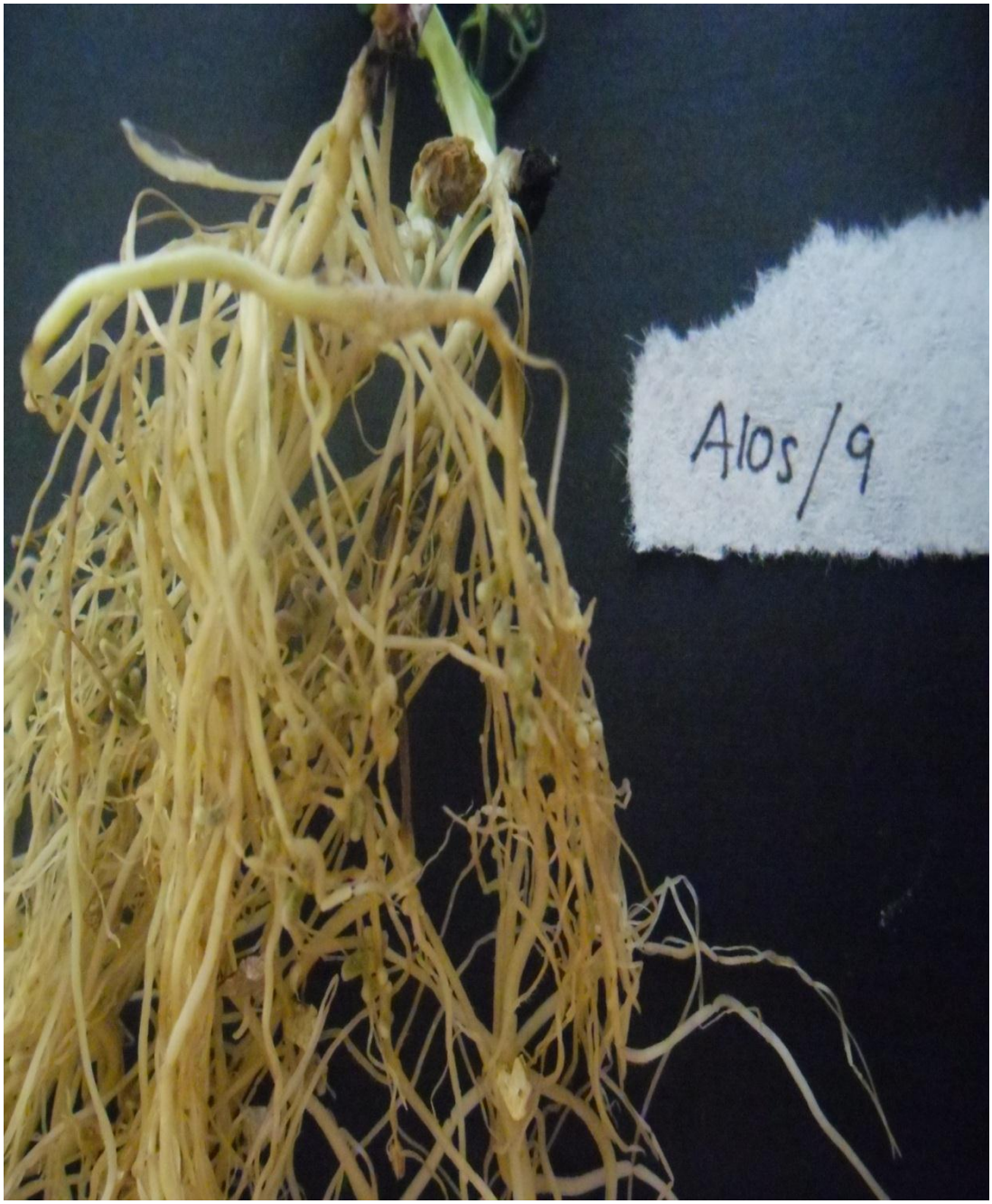

Plate 11: Nodulation parttern of peas under ALOSCA® granule treatment

The failure of nodule to form from *Rhizobium* inoculant for peas can be explained by unfavourable conditions such as high soil N, failure of effective *Rhizobium* to develop in the rhizosphere, plant agrochemical treatment, unsuitable inoculant application methods or a combination of these factors (Gibson and Pagan, 1977; Hamdi and Alaa El-Din, 1981; Fettell *et al*., 1997; Bollman and Vessey, 2006). The poor response of the pea plants to the inoculum bacteria may also be due to competition of other strains of bacteria and fungi. The bacterial strain, in the peat inoculant applied in the field trial was *Rhizobium leguminosarum*

bv. *viciae* (SU303). The strains in the ALOSCA® granules, applied in the field trial were *Rhizobium leguminosarum* bv. *viciae* (SU303 and WSM 1455). There was evidence of poor competition, of the strains, against indigenous soil bacteria, and the proportion of nodules of pea plants induced by strain SU303 was less than 15 %. The corresponding proportional presentation of inoculant rhizobia in pea root nodules varied from 63 - 79 % to less than 15 % and 5 % as soil existing populations of *Rhizobium leguminosarum viciae* was 30.537 and 16.980 g $soil^{-1}$ at different sites (Evans *et al*., 1996). Nodulation also occurred in control plots, in this experiment, which suggest indigenous *Rhizobium leguminosarum bv. viciae* was present at the experiment site and they competed with inoculated bacteria (Corbin *et al*., 1977; Fettell *et al*., 1997). Fettel *et al*. (1997) found that *Rhizobium legumonisarum* bv. *viciae* can exist in acid soils where pea has never been cropped. The survival or saprophytic competence of *Rhizobium* generally, or *Rhizobium leguminosarum* bv. *Viciae* particularly strongly depend on soil properties. It was concluded that in strongly acid soils with pH < 4.1, a two year absence, of a host crop, was sufficient to detect no *Rhizobium leguminosarum* bv. *Viciae.* However, in mildly acidic soils, *Rhizobium leguminosarum* bv. *Viciae* could be detected ten years after initial inoculation (Evans, 2005).

In addition, a satisfactory soil nitrogen level for the growth and development of peas is another possible reason for the poor response of peas to *Rhizobium* inoculation and nitrogen application. Soil nitrate has been shown to delay nodulation and decrease nodule number and activity (Herdina and Silsbury, 1989). Even when plants are well nodulated, the rate of N fixation is supressed in the presence of NO_3^- (Silsbury, 1984). The soil test result showed that the soil nitrate content in the experiment ranged from 35.5 to 54 kg ha^{-1} and this is considered sufficient to inhibit nodulation and nitrogen fixation (Voisin *et al*., 2002).

7.9.2 Nitrogen application response

In the field trial N application slightly increased nodulation of peas compared with the control (Table 6.1). However, this difference was not sufficient to enhance growth or final yield in the N application plots compared with control plots. This was because the soil nitrogen was sufficient to satisfy demand by the peas. This finding is similar to the result, obtained earlier, by Gan *et al*. (1997) who found that application of 25 kg N before sowing gave greater nodulation but had no effect on growth or final yield.

7.9.3 Sowing date response

In this experiment, plants from November sowing had a higher nodulation score than from October sowing (Table 6.1). This was because of a higher soil temperature, for nodule establishment, during early growth in November than in the October sowing. Nitrogen fixation and nodulation of legumes are sensitive to the environment and a low soil temperature is an important constraint to nodule formation and development in legumes (Lira *et al*., 2005). Low temperature delayed nodule initiation, significantly reduced nodulation, nodule growth rate, final nodule size, nodule activity and influenced the onset of nitrogenase activity (Schweitzer and Harper, 1980; Lira *et al*., 2005). In the field, a decrease in temperature from 20 °C to as low as 10 °C increased the time required for nodulation. The nodulation of pea plants was poorer at 10 °C than at 15 °C or 20 °C (Herdina and Silsbury, 1989). Peas showed their highest nodulation rate and largest nodules at a root temperature at 20 °C. In Sowing 1 plants were grown under low air temperatures of 11 °C during early growth compared with 14.3 °C and 16.8 °C in Sowings 2 and 3 (Figure 2). As a result, the corresponding soil temperatures were 12.3 °C, 16.5 °C and 19.5 °C, respectively. Therefore the nodulation rate of plants in Sowing date 1 was slower than in the later sowings and plants in Sowing 1 had lower nodulation scores.

Conclusions

1. Generally, the final seed yields, in the field trial were insufficient to give an economic benefit to growers in New Zealand from inoculation. However, field pea in an October sowing gave the highest seed yield at more than 4 t ha^{-1} and it was considered to economically viable in New Zealand. To achieve an economically viable final seed yield, a higher sowing rate, to achieve a target population of at least 100 plants m^{-2} is recommended. This is particularly important for the determinate, semi-leafless garden pea 'Ashton'.
2. *Rhizobium* inoculant had a statistically significant positive effect on the field nodulation of pea plants in the field. However, the nodulation score of all inoculated plants was lower than expected and the effect was insufficient to give differences in the TDM or final seed yield between inoculum treatments and the control.
3. Starter N fertilizer had no effect on nodulation, TDM or seed yield in this study.
4. 'Aragorn' had a greater ability to maintain seed yield than 'Ashton' when they were grown at a lower than optimum plant population. After severe bird damage, 'Aragorn' compensated for a very low plant population by producing more pods $plant^{-1}$ and an increased thousand seed weight.

Further studies

1. The competitiveness of *Rhizobium* strain SU303, compared to soil existing bacteria, and factors that affect the viability and effectiveness of the bacterial strain should be clarified.
2. The total amount of N fixed estimated by appropriate method such as N^{15} from the symbiotic relationship between peas and *Rhizobium* strain SU303 should be measured in Canterbury.
3. The response of peas to different starter N doses in terms of nodulation, N fixation, growth and development will help to find the answer about the effect of starter N on pea plant growth in New Zealand.

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Appendices

Appendix 1: TDM accumulation (t ha^{-1}) at different sampling times of 'Ashton' and 'Aragorn' peas under different nitrogen sources in Canterbury in 2010/2011 growing season.

Treatment	Ashton-Control	Ashton-50 kg N	Ashton-Peat	Ashton-ALOSCA	Aragorn-Control	Aragorn-50 kg N	Aragorn-Peat	Aragorn-ALOSCA
45 DAS	1.48	1.58	1.46	1.56	1.51	1.52	1.74	1.78
60 DAS	5.58	6.65	5.08	5.9	5.23	6.32	7.05	6.16
75 DAS	7.13	7.29	6.53	7.16	8.27	8.45	9.86	7.94
SEM	0.561							
Significance	ns							
CV%	18.7							

Appendix 2: Final seed yield of 'Aragorn' and 'Ashton' under different nitrogen source in Canterbury, New Zealand in 2010/2011 growing season.

Cultivar	Nitrogen source			
	Control	50 kg N	Peat inoculant	ALOSCA granule
'Ashton'	3.47	3.89	3.60	3.63
'Aragorn'	4.36	3.89	3.87	3.78
SEM	0.342			
Significance	ns			
CV%	17.9			