EVALUATION OF GRANULAR *RHIZOBIUM* INOCULANT FOR CHICKPEA

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ABSTRACT

Legume seeds are usually inoculated with liquid or peat-based rhizobial inoculants, but the recent introduction of soil-applied granular inoculants for chickpea (*Cicer arietinum* L.) in Saskatchewan has stimulated interest in this formulation. Field and growth chamber experiments with chickpea were conducted to assess the efficacy of granular inoculants compared to seed-applied liquid or peat-based inoculants.

In the field, granular inoculants were either placed in the seed furrow or side banded 2.5 or 8.0 cm below the seed. The nodule dry weight for the liquid inoculant was lower than that for the peat or granular inoculants. Nodule formation in the seed-inoculation treatments was restricted to the crown region of the root system. whereas soil inoculation in particular, below the seeding depth resulted predominantly in lateral root nodules. In the field, soil inoculation increased dry matter yield plant^{-1} over seed inoculation, but the increase was minor in the growth chamber. In 1997 granular inoculant placed below seed increased kabuli seed yield by 36 and 14% over the liquid and peat-based inoculants, respectively, whereas desi seed yield increased 17 and 5%. respectively. However, yields were inconsistent in 1998. In the field. seed protein concentration, percentage N derived from atmosphere (%Ndfa) and amount of N_2 fixed for the seed were typically lower for the liquid inoculant than those for the peat and granular inoculants. Similar trend was observed for %Ndfa and N_2 fixed in the growth chamber. The rate of N_2 fixation in the growth chamber increased from the late vegetative stage (28 DAP) to a peak at the early pod-filling stage (56 DAP) and declined thereafter. The dry weight of lateral root nodules was highly correlated with both plant dry weight and seed yield but the relationship was inconsistent in kabuli in 1998, presumably due to droughty conditions. Based on the field results. placing granular inoculant 2.5 to 8.0 cm below the seed may be the optimum.

The isotopic fractionation (β) values during N_2 fixation by desi and kabuli chickpeas, grown in N-free nutrient solution, were not influenced by the infecting rhizobial strain at the flowering stage, but the β values for the harvested seed in the

desi were dependent on the rhizobial strain. Nodule dry weight, plant dry weight and N accumulation did not differ in either the desi or kabuli chickpea, except for plant N yield, which was lower in the mixed-strain inoculant in the kabuli chickpea.

The survival of *Rhizobium ciceri* on chickpea seed, treated separately with Apron, Arrest 75W, Crown or Captan, was examined under laboratory conditions Fungicide treatment decreased rhizobial viability on the seed. The toxicity of the fungicides in terms of rhizobial viability increased in the following order: Control = Crown < Arrest = Apron < Captan. In the growth chamber, Crown reduced nodulation, N_2 fixation and shoot dry matter. Seed treated with Arrest and Captan decreased nodule dry weight and N_2 fixation, but only Arrest reduced dry matter yield. Apron had no effect on any of the parameters measured at the early pod-filling stage and may be compatible with chickpea inoculum.

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1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important dryland pulse crops in the Indian sub-continent, Turkey and the Middle East. It has recently been introduced into Saskatchewan and currently is grown on nearly 140,000 ha (Saskatchewan Agriculture and Food, 2000). Like other legumes, chickpea can fix atmospheric N_2 through a symbiotic association with an effective strain of *Rhizobium*, reducing its dependence on soil N. The chickpea-*Rhizobium* symbiosis is highly specific (Silsbury, 1989), and because western Canadian soils do not contain sufficient numbers or the specific rhizobia to establish an effective association, inoculation is essential to ensure that a large and effective rhizobial population is available in the rhizosphere of the plant to facilitate nodulation and N_2 fixation (Hynes et al., 1995).

The success of any inoculation program depends on many factors, including environmental conditions, rhizobial strain, inoculant carrier and inoculation method (Smith, 1992; Hynes et al., 1995). Most early research in the area of *Rhizobium* inoculant formulation focused on the carrier material, which included peat (Kremer and Peterson, 1982); coal (Crawford and Berryhill, 1983); clay, e.g., montmorillonite and vermiculite (Sparrow and Ham, 1983; Paau et al., 1990); alginate (Jung et al., 1982); polyacrylamide gel (Dommergues et al., 1979); and compost made from sawdust or rice husks (Khatri et al., 1973). Ideally, the carrier material should support large numbers of viable rhizobia for extended periods of time in a suitable physiological state to maintain the effectiveness of the rhizobia and to facilitate the ready formation of a symbiotic association with the host seedling (Paau et al., 1990; Paau, 1991).

The most common inoculation method involves treating the seed with a peat-based or liquid inoculant prior to planting. Although this practice is widely accepted, its efficiency is questionable under several situations (Brockwell and Bottomley, 1995; Brockwell et al., 1995). The following have been identified as situations or conditions

in which seed inoculation may not be suitable: (1) pre-emergence disease or insect attack may make it necessary to use seed dressings of fungicides or insecticides, many of which are toxic to rhizobia (Brockwell, 1977; Brockwell and Bottomley, 1995; Brockwell et al., 1995); (2) inoculation for large-hectare sowings of pulse crops with high seeding rates is a major task, which restricts the seeding operation (Brockwell, 1977; Prairie Agricultural Machinery Institute, 1991; Rennie et al., 1993); (3) seeds of crops, which push the seed coat and the cotyledons out of the soil during emergence (epigeal emergence), in which case rhizobia on the seed coat are not deposited in the soil (Brockwell, 1977; Jauhri and Rao, 1989); (4) seed coats of some legumes contain materials toxic to rhizobia (Thompson, 1960); (5) some seeds are extremely fragile and over-handling can cause reduced germination and emergence (Wani et al., 1995); (6) the seed surface places a limit on the number of rhizobia which may be applied, a common problem when seed size is small (Brockwell et al., 1980; Clayton et al., 1996); and (7) there is little protection from desiccation on the seed before planting and exposure to environmental stresses, including drought and high temperature after planting (Kremer and Peterson, 1982; Smith, 1992).

As a consequence of the many limitations associated with seed inoculation, interest is growing in the use of granular inoculants because they are applied directly to the soil. Granular inoculation has advantages in terms of storage, handling and ease of application and the fact that rhizobial rates can be increased far beyond those applied by conventional seed inoculation (Bezdicek et al., 1978). Soil inoculation minimizes direct contact with chemically treated seed and does not involve seed mixing which may disrupt delicate seeds (Smith, 1992). Granular inoculants are able to withstand low moisture conditions as compared to the powdered form (Dean and Clark, 1977). Furthermore, granular inoculant provides slow release of rhizobia over a longer period (Bashan, 1986). Although the superiority of direct soil inoculation over seed inoculation is widely recognised, little information is available on this method of introducing rhizobia to the soil. Therefore, the main objectives of this research program were to:

1. Evaluate the effect of seed and soil inoculation methods on nodulation, N_2 fixation and yield in chickpea;
2. Determine the optimum placement depth for granular inoculum;
3. Examine the contribution of lateral root nodulation to N_2 fixation and yield;
4. Investigate the time-course of N_2 fixation in chickpea;
5. Examine the survival of *Rhizobium ciceri* strain CP39 inoculated onto fungicide-treated chickpea seeds, and the subsequent nodulation, N_2 fixation and dry matter production of chickpea.

2. LITERATURE REVIEW

2.1 Chickpea

Chickpea belongs to the family Leguminosae, subfamily Papilionoideae, and tribe Viceae (Saxena, 1984). It is an indeterminate herbaceous annual. According to Singh (1978), the likely progenitor of the cultivated species is *Cicer reticulatum*. The main cultivated types are the large-seeded, rounded and cream-coloured kabuli chickpea (also known as garbanzo), and the relatively small-seeded, irregularly shaped and variously coloured desi chickpea, also known as bengal gram (Smithson et al., 1985). As a result of its larger size and reduced pigmentation (tannin), the kabuli chickpea is regarded as more advanced through sustained selection (Smartt, 1990). Although loss of pigmentation improves the nutritional quality of chickpea, it increases susceptibility to insects and diseases. For this reason, the distribution of the two types may be related in part to the distribution and severity of insects and diseases.

The cultivated form of chickpea likely originated in Anatolia, Turkey (Ladizinsky, 1975; Keatinge et al., 1995) and traditionally has been grown throughout the semi-arid regions of the Indian sub-continent and the Mediterranean (Singh and Auckland, 1975). It is the third most important pulse crop (after dry bean and pea), accounting for about 15% of the world pulse production (Saskatchewan Pulse Crop Development Board, 1997). India, Pakistan, Bangladesh and Nepal grow almost 90% of the total world area of chickpea (Minchin et al., 1980; Saxena, 1984). India is the largest chickpea producer with an annual production of about 4.5 million tonnes from about 7.0 million ha (Amin et al., 1994), and Turkey is the largest chickpea exporter (Keatinge et al., 1995).

Chickpea is also an important crop in Mexico. In Australia and North America, chickpea is a recent introduction. The first commercial cultivation of the kabuli chickpea in the United States began in 1981 and it is now grown in California and in

the Palouse Region of Washington and northern Idaho (Kaiser and Muehlbauer. 1994). In Canada, both desi and kabuli chickpeas were introduced into the western Canadian agricultural system in the late 1980s (Vandenberg and Slinkard, 1996). The crop is best suited to the Brown and Dark Brown soil zones (Vandenberg and Slinkard, 1997).

Chickpea is a cool season plant usually grown as a winter crop in India, the Middle East, Australia, and South and Central America and matures on residual soil moisture. It is very sensitive to excessive moisture, high humidity and cloudy weather which limit flower production, seed set and yield (Kay, 1979), but increase the incidence of diseases (Saxena, 1984). Among the four major diseases (ascochyta blight, fusarium wilt, botrytis and stunt) of chickpea, ascochyta blight is the most serious and can destroy the entire crop (Smithson et al., 1985; Saskatchewan Pulse Crop Development Board, 1997).

2.2 Symbiotic nitrogen fixation

Chickpea, like most legumes, establishes a symbiotic association with a compatible strain of *Rhizobium*. The *Rhizobium*-legume symbiosis is a well-organized system involving many steps: signal exchange and recognition of the symbiotic partners; attachment of the rhizobia to the plant root hairs; root hair deformation: invasion of the root hair by rhizobia; infection thread formation; nodule initiation; bacteriod development; and formation of N_2-fixing nodules (e.g., Vincent, 1980; Sprent, 1989; Hirsch, 1992; Mylona et al., 1995). Nodules are grouped into two main types; determinate and indeterminate (Hansen, 1994). In determine nodules [e.g., soybean (*Glycine max* L. Merr.), common bean (*Phaseolus vulgaris* L.)], cell division is over a short duration and the nodules are usually spherical. In contrast, indeterminate nodules [e.g., pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.)] possess a meristem which gives rise to differentiated cells that may become infected with rhizobia. Due to the continued cell division indeterminate nodules are generally cylindrical in shape (Hansen, 1994). Chickpea nodules have not been studied in detailed but based on the shape, they may be indeterminate.

Once symbiosis is established the host plant provides carbon substrate as a source of energy, and the bacteria reduce atmospheric N_2 to ammonia, which is exported to plant tissues for protein synthesis (Keyser and Li, 1992, Paul and Clark, 1996). The effectiveness and efficiency of the symbiotic system is dependent markedly on the mutual compatibility of both partners (Keyser and Li, 1992). Thus, in many soils, sufficient numbers of the bacteria of the correct rhizobial species, and strain for the host cultivar must be introduced (Hynes et al., 1995). Despite the selection of effective rhizobial strains for use as inoculants, inoculation does not always lead to increased N_2 fixation due to environmental stress and the inability of the inoculant strain to occupy a significant proportion of the nodules (McLoughlin et al., 1990a,b; Thies et al., 1991; Griffith and Roughley, 1992; Carter et al., 1995; Issa and Wood, 1995).

2.3 Factors influencing the success of inoculation

The success or failure of an inoculation technology is determined by a number of factors. Soil factors, such as moisture (Boonkerd and Weaver, 1982; Postma and van Veen, 1990; Griffith and Roughley, 1992; Issa and Wood, 1995), temperature (Munevar and Wollum, 1981; Roughley, 1985; Kluson et al., 1986), pH (Evans et al., 1990; Blamey et al., 1993; Flis et al., 1993; Brady et al., 1994), salinity (Singleton et al., 1982; Singleton, 1983; Elsheikh and Wood, 1990a,b; Zahran, 1991), N availability (Streeter, 1988; Minchin et al., 1989; Abaidoo et al., 1990; Kanayama, 1990), climatic conditions (Roughley et al., 1993; Hansen, 1994), and the presence of competing indigeneous rhizobial populations (Bohlool and Schmidt, 1973; Singleton and Tavares, 1986; Thies et al., 1991, 1992), influence the ability to achieve increased crop productivity through inoculation. Under adverse climatic or soil conditions or when indigeneous rhizobial populations are high, soil inoculation out-performed the conventional seed-applied inoculant (Scudder, 1975; Dean and Clark, 1977; Bezdicek et al., 1978; Brockwell et al., 1980; Kamicker and Brill, 1987; Danso and Bowen, 1989; Hardarson et al., 1989; McDermoutt and Graham, 1989; Danso et al., 1990; Rice and Olsen, 1992). In these studies, the significance of high rates of inoculum in

achieving maximum survival of the introduced rhizobia was emphasized. The success of soil inoculation in the field depends on the relative competitive advantage provided by the high rate of rhizobia application and the ability of the rhizobia to persist under unfavourable environmental conditions when applied as granular inoculant as compared to seed-applied inoculation.

2.3.1 Effect of the inoculated seed

For any inoculation method, the number of rhizobia applied and the number that survive are important factors that influence nodulation and N_2 fixation. Evaluation of seed-applied inoculation has revealed that in small seeded legumes the surface area of the seed often cannot accommodate sufficient inoculant to obtain maximum nodulation (Brockwell et al., 1980). In addition, the numbers of infective rhizobia can drop dramatically between seed inoculation and planting (Rodriguez-Navarro et al., 1991; Ramos and Ribeiro, 1993; Roughley et al., 1993). In these studies, poor survival of inoculant rhizobia after their application to the seed was linked to seed coat toxins, chemical treatments and other environmental factors.

2.3.1.1 Seed size

The inoculum potential of seed-applied inoculants is a function of the number of rhizobia applied and their subsequent survival both on the seed and before germination. In studies on the effect of inoculant rates on nodulation, the numbers of rhizobia and quantities of carrier have been confounded (Roughley et al., 1993), making it difficult to separate the individual effects. One important disadvantage of seed-applied inoculants is the limitation of the quantity of rhizobial inoculum that can be placed on the surface of the seed (Brockwell et al., 1980, 1982; Smith, 1992; Clayton et al., 1996). Although high rhizobia populations can be easily applied to large-seeded legumes (e.g., kabuli chickpea), the scope for this approach is limited because a large amount of the inoculant on the seed is unlikely to remain in place during passage through the seeder (Brockwell et al., 1988; Roughley et al., 1993). According to Brockwell et al. (1988), inoculant losses in the range of 94-99% occurred

between soybean inoculation and planting, attributable, in part, to separation of inoculant and seed as it passed through the machinery. On the contrary, they observed no such loss of rhizobial viability with liquid inoculants applied directly to the seedbed.

2.3.1.2 Mode of seed germination

Another downside of seed-applied inoculants is that in legumes with epigeal germination the seed coat often adheres to the cotyledons when they are pushed above ground during seedling emergence, leaving only a portion of the inoculum in the soil. In the case of crops grown on residual soil moisture, the introduced rhizobia cannot move downward with the growing root from the dry surface soil where the inoculum was placed (Wani et al., 1995). The only report on the influence of epigeal germination on inoculation is by Jauhri and Rao (1989), who evaluated the reduction in the inoculated rhizobial population due to epigeal germination and emergence in soybean (*Glycine max*) seed, using different levels of gum arabic as adhesive. They found that the loss of rhizobia increased linearly with increasing concentration of gum arabic and decreased with increase in soil moisture or the depth of placement of inoculated seed in the moist soil. The results suggest that increase concentration of gum arabic enhanced the binding of the inoculant rhizobia to the seed coat that eventually was carried above the soil surface. On the other hand, an increase in soil moisture as well as depth of sowing facilitated release of the rhizobia from the seed coat.

2.3.1.3 Seed-coat toxins

Toxic diffusates from seed coats affect the survival of rhizobial inoculum applied to legume seeds (Thompson, 1960; Materon and Weaver, 1984; Rodriguez-Navarro et al., 1991). Thompson (1960) showed that untreated and autoclaved seeds of subterranean clover (*Trifolium subterraneum* L.) and their extracts inhibited the growth of *Rhizobium trifolii* when placed on the surface of yeast mannitol agar in petri dishes with a suspension of 5×10^5 rhizobia, but soaked seed did not. Further investigation showed that the inhibitor was associated with, and extractable from, the seed coat. The presence of inhibitory compounds in the seed coat of subterranean

clover was evident since soaking the seed before inoculation and planting greatly improved nodulation by the applied inoculum. Furthermore, physical separation of the seed coat and the inoculum, by coating the seed with inert material before inoculation, improved nodulation of subterranean clover (Thompson, 1960).

Bowen (1961) tested surfaced-sterilized seed of *Centrosema pubescens*, subterranean clover and alfalfa (*Medicago sativa* L.) against seven *Rhizobium* strains isolated from a wide range of legumes. He found that the degree of inhibition varied markedly with *Rhizobium* strain and legume species. Generally, seed diffusates from subterranean clover were more inhibitory than those from *C. pubescens* or alfalfa. Moreover, almost all of the antibacterial activity arose from the seed coat. The relationship between the inhibitory effect, identified by the agar-plate assay, and the multiplication of *Rhizobium* around the seeds in a more natural environment was studied by inoculating subterranean clover seeds and planting them in heat-sterilized sand or soil moistened to field capacity with plant nutrient solution. He found that *R. trifolii* strain RTR 151 multiplied on seed in sand, but did so to a much lesser extent than on glass beads used as the control. In the soil, a decline in population occurred around the seed, whereas a slight increase occurred around the beads. He concluded that seed diffusates had an inhibitory effect on rhizobial growth.

The inhibitory effect of seed diffusates of different legumes on rhizobial growth was also examined by placing surfaced-sterilized, soaked and unsoaked seeds in petri plates on which a rhizobial population had been established (Dadarwal and Sen, 1973). The unsoaked seeds of all the legumes examined showed a clear growth inhibition zone around them, but the soaked seeds were not inhibitory. In addition, Dadarwal and Sen (1973) investigated the survival of rhizobia inoculated on surface-sterilized soaked (for 24 h) and unsoaked pea (*Pisum sativum*) and desi chickpea seeds. For the unsoaked seeds, the applied rhizobial population declined by 40 and 88% for the pea and desi chickpea, respectively, after 24 h. Seven days after inoculation, only 10 and 6% of the initial numbers of rhizobia applied to the unsoaked pea and desi chickpea seeds, respectively, survived. In contrast, the rhizobial population on the soaked seeds increased over the first seven days. In pot studies, they

found that inoculation of unsoaked seeds increased pea yield by 28.7% and desi chickpea yield by 33.8%, whereas inoculation of soaked seeds increased pea yield by 79.5% and desi chickpea yield by 74.5%.

Rodriguez-Navarro et al. (1991) observed that the failure in the establishment in a new sulla (*Hedysarum coronarium* L.) field was associated with a decrease of viable rhizobia on the seeds before they germinated. The decline in viability was attributed partially to seed coat toxicity. Similarly, Materon and Weaver (1984) reported a toxic seed coat effect on *Rhizobium* populations. For example, a 10-fold decline in rhizobial numbers within one day was found for *R. trifolii* peat inoculant on white clover (*Trifolium repens* L.) seeds (Materon and Weaver, 1985). A 90% reduction in the number of viable cells of *R. meliloti* and *R. trifolii* occurred within one hour when peat-base inoculant was applied to alfalfa and white clover seeds (Burton, 1976). Similarly, significant losses of viability of *B. japonicum* peat inoculum on soybean seeds were observed by other researchers. In these studies, a 10-fold decline was observed after one week (Davidson and Reuszer, 1978), after two days (Elegba and Rennie, 1984) and after one hour (Burton, 1976).

The nodulation failure of birdsfoot trefoil (*Lotus corniculatus*) was attributed to a rapid decline in numbers of viable rhizobia on the seed due to seed coat toxicity, as only 5% of those applied were present 24 h after inoculation (Chapman et al., 1990). Similarly, Lowther and Patrick (1995) observed that the survival of 15 strains of *Rhizobium loti* on birdsfoot trefoil seed 24 h after inoculation varied from 1 to 89%. Working with *R. leguminosarum* bv. *trifolii* strains WU95, *Bradyrhizobium japonicum* strain CB1809 and *B. lupini* strain WU425, Griffith and Roughley (1992) reported that numbers of viable rhizobia on seed dropped rapidly in the first 6 h, whereas on beads some multiplication occurred up to the third day. Thereafter, numbers declined with time, but were always significantly greater on beads than on seeds for the first 14 d after inoculation. After storage for 28 d, this difference in survival disappeared. While the rapid death rate on seeds compared with beads in the first 6 h after inoculation could be attributed to the effects of seed coat toxin, the effect of environmental stress, such as desiccation, could have been a complicating factor.

2.3.2 Effect of environmental factors

The environmental conditions during inoculation and planting can affect the survival and infectivity of rhizobia on the legume seed. Dehydration of inoculated seed and its exposure to high temperature have been identified as major factors limiting nodulation success (Brockwell et al., 1987; Roughley et al., 1993; Hansen, 1994).

2.3.2.1 Moisture

Inoculant carriers help stick the inoculum onto the seed surface and protect the rhizobia, to some extent, from desiccation. However, desiccation of the seeds and adhering rhizobia is still a serious problem, when using conventional inoculation techniques (Hansen, 1994). Only a few studies have been conducted to examine the effect of dehydration on viability of rhizobia after seed inoculation and before planting. Roughley et al. (1993) determined the survival of *Bradyrhizobium sp.* on narrow-leaf lupin *(Lupinus angustifolius* L.*)* during seed inoculation, transport to the field, planting and on seed recovered from the soil. Using the most probable number (MPN) method, they found that the number of viable bradyrhizobia declined by a factor of 10 after one hour. During the 3.75 h that elapsed, while the seed was augered into a truck from the mixer, transported to the field and augered into the seed box, the number of viable rhizobia declined to less than 1% of the original number. Rapid death occurred in the air seeder where a further decline of 40% occurred in 5 min. At the point of sowing, the number of viable rhizobia per seed decreased from $\log_{10}$ 5.15 to 3.83, an overall loss of 1.3×10^5 rhizobia or 95% of the rate applied. Following the first day in the soil, 85% of the remaining rhizobia died as a result of desiccation. The quantity of peat within the range of 0.125 - 3 times the Australian recommendation for inoculating seed had no effect on the nodulation of narrow-leaf lupin, indicating the carrier offered the rhizobia little or no protection from desiccation.

The decline in viability of *R. trifolii* strains WU1 and *R. meliloti* WU96 was investigated during the first hour after inoculation of mung bean (*Vigna radiata*) seeds (Salema et al., 1982). Besides using adhesive alone for the inoculation, they included a treatment in which a mixture of sucrose and sodium glutamate was added to minimize

desiccation of the rhizobial cells. The results indicated that, when the rhizobia on the seed were unprotected, the decline in numbers occurred in distinct phases: a phase of relatively slow death rate, while the seed remained moist, followed by a very high death rate phase shortly after loss of visible moisture on the seed. Following the second phase, numbers of viable rhizobia stabilized for about 15 min before a significant death rate resumed. On the other hand, the overall death rate was reduced when the rhizobia were protected against desiccation.

Tolerance to desiccation varies considerably among rhizobia. For example, slow-growing strains of *Bradyrhizobium japonicum* and the "cowpea miscellany" survived better than fast-growing *R. meliloti* and *R. trifolii*, when subjected to severe desiccation at 27°C or 50°C (van Rensburg and Strijdom, 1980). In contrast, a higher survival rate was recorded for the fast-growing strains than the slow-growing strains when subjected to mild desiccation (moisture tension of about 80 MPa) at 27°C. However, at this same moisture tension, the slow-growing strains survived in higher numbers than the fast-growing strains, when the temperature was increased to 40°C. Although the authors attributed the difference in behaviour of the *Rhizobium* species at different moisture tensions to differences in the internal water-retaining abilities of the cells, the fact that the slow-growing strains were more resistant to desiccation than the fast-growing strains at 40°C, but not at 27°C, illustrates that temperature also plays an important role in determining the survival of these *Rhizobium* strains on inoculated seed.

2.3.2.2 Temperature

Exposure of rhizobial inoculant to high temperatures during transportation, storage, and planting often results in decreased numbers and N_2-fixing effectiveness of the rhizobia (Ayanaba, 1977; Kremer and Peterson, 1983). In the tropics and subtropics, where high temperatures prevail during and after planting, poor survival of rhizobia in peat-based inoculants applied to seed is common (Scudder, 1975; Kremer and Peterson, 1982, 1983). For most rhizobia, the optimum temperature for growth in culture is between 28 and 31°C, with many unable to grow below 10 or above 37°C

(Graham, 1992). Somasegaran et al. (1984) reported a decline in viability of 10 inoculant strains during 8 weeks incubation at 37°C, while exposure to 46°C was lethal to all strains in less than 2 weeks. Storage of cowpea rhizobia in peat-based, seed-applied inoculant at 35°C also decreased root infection (Wilson and Tang, 1980).

The effect of temperature on the survival of rhizobia in soils has been extensively studied, but only a few researchers have examined the impact of excessive heat on rhizobia inoculated onto seed before planting. Inoculation of several legumes with different strains of rhizobia showed that rhizobial survival was better at 25°C than at 35°C after 2, 7 and 28 d following inoculation (Herridge and Roughley, 1974). Brockwell et al. (1987) reported that 99.9% of *B. japonicum* on seed died between inoculation and the time the seed was planted, and attributed this to the high air temperature of 38°C. For these reasons, rhizobia in granular inoculant with the rhizobial cells entrapped in the carrier and passed through several hardening treatments (Bashan, 1986) should be able to withstand harsh environmental stresses.

It is clear that significant losses of rhizobial cells can occur after seed inoculation and planting, decreasing the number of viable rhizobia available for nodulation. Since the number of viable rhizobia in the inoculum has an influence on nodulation and seed yield, these losses must be considered a possible limiting factor in inoculation and underscore the significance of an alternative method of inoculation to ensure the availability of sufficient numbers of rhizobia for effective nodulation.

2.3.3 Effect of fungicide seed treatment

Seed treatment with fungicide is essential in the production of many legumes to prevent losses from seedborne pathogens and seedling damping-off (Brockwell et al., 1980; Phipps, 1984; Sinclair and Backman, 1989; Ramos and Ribeiro, 1993). Although some reports are conflicting, a number of studies have conclusively shown that some of these chemicals are incompatible with *Rhizobium* (Rennie and Dubetz, 1984; Ramos and Ribeiro, 1993; Revellin et al., 1993).

Ramos and Ribeiro (1993) used five fungicides: Benlate 50% [(methyl-1-butylcarbomoil)-2-benzimidazolcarbamate], Vitavax 75% (5,6 dihydro-2 methyl-1,4-oxathiin-3- carbaxanilide), Banrot 40% [3-(2-methylpiperidino)-propyl - 3,4-dichlorobenzoate)], Difolatan 80% [cis-N-(1,1,2,2- tetrachloroethytrio)-4-cyclohexane-1,2-dicarboximide] and Ridomil 25% [alpha-(2 chlorophenyl)- alpha-4 (chlorophenyl)-5-pyrimidinemethanol] to evaluate fungicide effects on survival of *Rhizobium* on the seeds and subsequent nodulation of bean (*Phaseolus vulgaris* L). They found that these fungicides had deleterious effects on rhizobial survival 24 h after fungicidal seed treatment. Furthermore, they observed that under field conditions Benlate seed treatment with seed inoculation resulted in reduced nodule occupancy, whereas Benlate seed treatment with inoculant applied in the seed furrow had no effect on survival of the inoculum.

Curley and Burton (1975) found that Captan (N-tri-chloromethylthio-4-cyclohexene-1,2-dicarboximide) at 0.8 g kg^{-1} seed significantly reduced the number of the rhizobia after 24 h incubation. In a pot experiment, Chamber and Montes (1982) also observed that Captan at 2.0 g kg^{-1} seed reduced nodule mass and acetylene-reducing activity, when *B. japonicum* was either seed-applied or applied as a granular inoculant. However, they found that the number of nodules per plant was higher with granular inoculation than with seed-applied inoculation. Although Captan did not affect seed yield in this study, protein concentration was lower, particularly with the seed-applied inoculant. Rennie and Dubetz (1984), in a two-year field study, concluded that Captan, Thiram [bis(dimethylthiocarbamoyl)disulfide] and Carbathiin (5,6-dihydro 2-methyl-1,4-oxathiin-3-carboxanilide) had no effect on nodulation and N_2 fixation when granular inoculant was applied. In other studies, Thiram at 0.6 g kg^{-1} seed had no effect on numbers of viable rhizobia on the seed (Curley and Burton, 1975), but at 0.93 g kg^{-1} seed, it inhibited growth of *B. japonicum* (Tu, 1980, 1982) and reduced nodule mass and acetylene reduction activity over the entire seven weeks of a pot experiment (Tu, 1981). In contrast, Welty et al. (1988) observed that Thiram increased nodule weight and yield of chickpea.

Catroux and Arnaud (1991) showed that Carbendazim (methyl benzimidazol-2-yl carbamate) decreased the survival of *B. japonicum* on soybean seeds and also decreased nodulation and yield in the field, although early nodulation in the greenhouse was not affected. Similarly, Carboxin (5,6-dihydro 2-methyl-1,4-oxathiin-3-carboxanilide) decreased the number and weight of nodules and growth of soybean in pot experiments (Curley and Burton, 1975; Mallik and Tesfai, 1985; Tesfai and Mallik, 1986). However, in a mixture with Thiram, Carboxin had no effect on chickpea nodulation (Welty et al., 1988). Iprodione [3-(3,5 dichlorophenyl)-N-isopropyl-2,4-dioxoimidazolidine-1-carboxamide] also decreased the survival of *B. japonicum* (Evans et al., 1989), nodulation of lupins (Evans et al., 1986) and also decreased nodulation and yield of soybean in the field (Catroux and Arnaud, 1991). Revellin et al. (1993) reported decreased survival of *B. japonicum* and reduced nodulation and yield of soybean in both greenhouse and field studies using Germipro UFB (carbendazim and iprodione), Apron 35 J {metalaxyl [methyl N-(2 methyoxyacetyl-1-cyclopentyl)-3-phenylurea]}, and Tachigaren [hymexazol (5-methylisoxazol-3-ol)].

From the above discussion, it is clear that the deleterious effect of fungicides on inoculum is a consequence of the direct contact of the fungicide and inoculant when the latter is seed-applied. Therefore, granular inoculant, which avoids direct contact of the inoculant with the fungicide, may overcome the incompatibility problem between rhizobia strains and fungicides (Brockwell et al., 1980; Chamber and Montes, 1982; Rennie and Dubetz, 1984; Ramos and Rebeiro, 1993; Hansen, 1994).

2.3.4 Effect of soil factors

Soil environmental factors influence legume inoculation directly by affecting the multiplication, survival and distribution of the inoculant rhizobia in the soil and indirectly through their effects on the host plant. Thus, soil conditions can influence various stages of the nodulation process, such as rhizobial attachment, infection and nodule formation (Vlassak and Vanderleyden, 1997). The major limiting factors may

vary with location, but include moisture stress, high temperature, soil acidity and high available soil nitrogen (Graham, 1985).

2.3.4.1 Soil moisture stress

Soil water affects the number of introduced rhizobia in the soil, their distribution down the soil profile and the susceptibility of the plant root hairs to infection (Roughley, 1985). Gray and Williams (1971) pointed out that most microorganisms cannot multiply at matric potentials less than -1.5 MPa, due to their inability to exert sufficient suction to empty pores of less than 0.2 μm dia (the maximum diameter of water-filled pores at matric potential -1.5 MPa). Similarly, Amara and Miller (1986) found that the number of *Rhizobium phaseoli* declined at matric potentials less than -1.5 MPa. Investigation on the population dynamics of 10 strains of *B. japonicum* in loamy sand at water potentials between -1.5 and -0.01MPa showed that numbers of all strains declined in proportion to the water content (Mahler and Wollum, 1980). They observed that the numbers of *B. japonicum* cells were between one and three orders of magnitude smaller under a matric potential of -1.5 MPa than at or near field capacity. In a comprehensive study on the effect of soil water potential on growth and survival of root nodule bacteria in peat culture and on seed, Griffith and Roughley (1992) observed that all strains (*R. leguminosarum* bv. *trifolii*, *B. japonicum* and *B. lupini*) survived best at water potentials of -0.01 MPa compared to -0.25 MPa and -1.0 MPa. Populations of chickpea and bean rhizobia were also higher at -0.03 MPa than at -1.5 MPa (Issa and Wood, 1995).

However, differences in drought susceptibility exist among species of *Rhizobium*. For example, Bushy and Marshall (1977) observed that fast-growing strains of *Rhizobium* declined by four orders of magnitude during drying of a sandy soil, but the slow-growing strains declined by only two orders of magnitude. Van Rensburg and Strijdom (1980) and Mary et al. (1994) also suggested that fast-growing rhizobia are more susceptible to extreme desiccation in soil than the slow-growing rhizobia, although milder desiccation had little effect on the fast-growing rhizobia relative to the slow-growing rhizobia.

Apart from survival and multiplication, water supply affects the movement of rhizobia in the soil. Since spatial distribution of introduced rhizobia in the soil is a major factor determining the onset and pattern of nodulation on legume roots (Worrall and Roughley, 1976; Date, 1991), restricted movement of rhizobia during drought would affect N_2-fixation indirectly. Griffin and Quail (1968) suggested that moving bacteria require a continous water pathway in soil pores with neck radii less than 1 to 1.5 μm, which represents a soil moisture potential of -0.09 MPa. Hamdi (1971) found that the downward movement of *R. trifolii* in soil is directly related to the amount of water applied. In laboratory studies, percolating water was a major factor affecting the dispersal of rhizobial inoculum (Breitenbeck et al., 1988; Worrall and Roughley, 1991). Thus, nodulation of legumes planted in partly dry soils will likely be affected, due to the failure of the inoculum to migrate away from the inoculated site. This effect has been observed in light-textured soil, where seed germination and root penetration occurred without nodule development, although large numbers of rhizobia from the seed-applied inoculant were recovered from the inoculation site (Brockwell and Whalley, 1970).

Although few of the studies presented above correlated the rhizobial survival and distribution with nodulation and N_2 fixation, it is well established that the greater the number of the introduced rhizobia the better the nodulation and N_2 fixation (Weaver and Frederick, 1974a,b). Athar and Johnson (1996) demonstrated that nodule occupancy by strains of *R. meliloti* declined from 57% to 38% when water potential decreased from -0.03 to -1.0 MPa. The number of nodules was reduced by 42% and 70% as water potential decreased from -0.03 to -0.5 MPa and from -0.5 to -1.0 MPa, respectively.

For the above reasons, Brockwell et al. (1987) suggested that high rates of inoculation should increase nodulation and N_2 fixation. In addition, placement of the inoculant rhizobia in the soil zone, where infectable foci on the seedling roots formed, should enhance nodulation and nodule occupancy. As legume plants age, their roots extend beyond the zone of inoculation, particularly when the inoculant is seed-applied.

However, the proportion of nodules occupied by the inoculant rhizobia would be low because rhizobial movement is restricted under low-moisture conditions.

In low moisture soils, Scudder (1975) obtained higher nodulation and N_2 fixation with granular inoculation as compared to seed-applied inoculation. When rhizobia are introduced into low-moisture soil by seed inoculation, they are likely to remain at the depth of seeding, and be subjected to wide fluctuations in moisture and temperature stresses, unless distributed down the soil profile by rain or irrigation (Roughley, 1985). Therefore, placement of granular inoculant below the seeding depth would partly overcome the limited rhizobial mobility (Vance and Graham, 1995) and also enhance survival of the introduced rhizobia because of better moisture conditions. Furthermore, granular inoculant (e.g. clay carriers) is in a dry solid state and is less susceptible to desiccation, increasing survival of the rhizobia (Jung et al., 1982; Kremer and Peterson, 1983; Sparrow and Ham, 1983; Materon and Weaver, 1985).

2.3.4.2 High soil temperature

High soil temperature influences the growth and survival of *Rhizobium* (Roughley, 1985), competition for nodule occupancy (Roughley et al., 1980; Kluson et al., 1986; Graham, 1992), nodulation and nodule activity (Munevar and Wollum, 1981; Kishinevsky et al., 1992). At 28°C, Brockwell et al. (1987) recovered 4-5% of the viable soybean inoculum from the soil 24 h after sowing, but less than 0.2% survived sowing at 38°C. Different species of *Rhizobium* and different strains of the same species differ in their susceptibility to temperature. For example, the optimum temperature for growth of *B. japonicum* ranged from 27.4 to 35.2°C (Munevar and Wollum, 1981), whereas cowpea strains evaluated by Eaglesham and Ayanaba (1984) grew well at 40°C. Cowpea strain 201 survived better than strains 3281, T-1 and TAL-309 at 35°C (Boonkerd and Weaver, 1982). However, in many soils, the impact of high temperature on rhizobial survival is determined by the interaction between soil moisture and soil texture. In general, the adverse effect of high temperature on rhizobial survival is more pronounced in soils with high water content (Chatel and Parker, 1973; Boonkerd and Weaver, 1982; Roughley, 1985).

Certain clays, such as bentonite, kaolinite and montmorillonite, protect rhizobia from death associated with drying and heat stress (Bushy and Marshall, 1977; Hartel and Alexander, 1984; Heijnen and van Veen, 1991; Heijnen et al., 1992; AbdelGadir and Alexander, 1997). Heijnen et al. (1992) suggested that a clay amendment to sandy soils improved the survival of rhizobia by increasing the protective micro-habitats available to the bacteria in the soil. Marshall (1964) found that clay amendment to *Rhizobium* inoculant prior to soil inoculation with peat-base inoculant protected root-nodule bacteria against high temperatures. AbdelGadir and Alexander (1997) modified the technique of Bashan (1986) and Smidsrod and Skjak-Braek (1990) to immobilize *R. leguminosarum* bv. *phaseoli* cells in montmorillonite and kaolinite in a study on the survival and infectivity under heat stress. They found that the immobilized cells survived well and grew, whereas free cells added to the soil died rapidly at 43°C. Moreover, the isolates, which survived 43°C, were effective at nodulating kidney bean.

No one has specifically compared the performance of seed-applied inoculation to soil inoculation with granular inoculant under high soil temperatures, but it can be argued that clay-based granular inoculants would result in improved survival. Already some of the commercial granular inoculants (e.g., MicroBio RhizoGen, Saskatoon, Canada) use clay-amended carrier materials.

The temperature at the surface of soils in the tropics and subtropics is often high and can cause rapid death of rhizobia. For example, the maximum temperature in sandy soils of Western Australia was 59°C at 1.3 cm and 47°C at 5.1-cm depth (Chatel and Parker, 1973). Day et al. (1978) counted the number of cowpea rhizobia in the profile of soils at Samaru, northern Nigeria, where bare soil surface temperatures can exceed 60°C. In the upper 5 cm, 5 to 50 rhizobial cells per g soil were present, and increased with depth, reaching 18,000 rhizobial cells per g soil at 20 to 25 cm. Thus, high temperatures can restrict rhizobial numbers and, consequently, nodulation to the subsurface region where temperatures are not extreme. Alfalfa plants grown in hot soil conditions in California formed few nodules in the top 5 cm of the soil, but nodulated extensively below this depth (Munns et al., 1977). In bean, Graham and Rosas (1978)

also reported fewer nodules close to the surface in spaced plantings than in plantings with closed canopies and attributed these differences to soil temperature. Consequently, a method of inoculation which places the inoculant rhizobia at an optimum depth would undoubtedly maximize the benefit from inoculation.

High soil temperature also influences the proportion of nodules formed by strains of *B. japonicum* from different serogroups (Weber and Miller, 1972). Roughley et al. (1980) found that strains of other *Rhizobium* species were poor competitors with *B. japonicum* on the promiscuously nodulating soybean cultivar Malayan between 24 and 33°C, but at 36°C they formed about 74 to 88% of the nodules. Graham (1992) suggested the use of higher than normal inoculation rates under such high temperature conditions. In Puerto Rico, Smith and del Roi Escurra (1982) reported that granular inoculant at about 10 times the normal application rate was required for good nodulation. In another study, a seed-applied treatment, providing log 0.59 cells cm^{-1}, was not successful in forming nodules, whereas granular inoculant treatments, that provided between log 5.59 and log 6.59, produced significant nodulation (Smith et al., 1981). Similarly, Wey and Saint Macary (1982) demonstrated maximum nodulation of soybean, when 10^{13} cells ha^{-1} of USDA 138 were applied as a granular inoculant in a hot tropical soil in Senegal.

2.3.4.3 Soil acidity

The influence of soil pH on the growth and survival of rhizobia is well documented (Graham, 1992; Jayasundara et al., 1998), but its influence on competition for nodule occupancy has received little attention. In general, nodulation declines at soil pH below 5.0 in most species including lupin, which is regarded as relatively acid tolerant (Jayasundara et al., 1998). For inoculated legumes in low pH soils, problems often include death or failure of the inoculant strain to multiply, due to H^+, Mn^{2+} or Al^{3+} toxicity, and deficiencies of Ca, Mg or P (Coventry et al., 1987; O'Hara et al., 1988; Richardson and Simpson, 1988; Evans et al., 1990, 1993; Carter et al., 1995), inhibition of root hair growth and infection (Flis et al., 1993) and inhibition of nodule

functioning through reduced availability of molybdenum (Coventry et al., 1985; Rai, 1991; Blamey et al., 1993; Brady et al., 1994). How these factors interact is not clear.

Differences among rhizobial strains in pH tolerance alter the outcome of competition among strains. For example, Voss et al. (1984) found that nodule occupancy of the bean strains Car37 and Car43 was reduced from 22 and 65%, respectively, in soil of pH 5.1, to only 3 and 5% after the soil was limed to pH 6.7. On the other hand, nodule occupancy by Car04 increased from 12% at pH 5.1 to 60% at pH 6.2. Similar results have been reported by others (Dughri and Bottomley, 1983; Ramos and Boddey, 1987; Vargas and Graham, 1988).

Several approaches have been used to increase nodulation when rhizobia are used in acid soils (Vance and Graham, 1995). These include liming the soil, which is expensive for low resource farmers in the tropics, and pelleting the seed with lime. Although the latter technique is relatively inexpensive, it can interfere with planting operations.

Increased inoculum rates have enhanced nodulation response in some studies (Munns, 1968; Pijnenborg et al., 1991). On soil with pH 5.8, granular inoculant, applied with or below the alfalfa seed, produced more nodules with nodule occupancy between 87 and 98% compared to the seed-applied treatment which had a nodule occupancy of 49% (Rice and Olsen, 1988). The authors reported similar results in another experiment conducted at the same location in Alberta (Rice and Olsen, 1992). Thus, soil inoculation, using granular inoculant, is one effective way to improve inoculation response in acid soils.

2.3.4.4 High available soil nitrogen

High levels of combined N inhibit root infection, nodule initiation, and nodule development and function (Keyser and Li, 1992; Dogra and Dudeja, 1993; Biederbeck et al., 1996), but the precise mechanisms responsible for the inhibitory effects are poorly understood (Streeter, 1988). However, the effect varies with the host plant (Chalifour and Nelson, 1987), the inoculant strain (McNeil, 1982; Gibson and Harper, 1985; La Favre and Eaglesham, 1987) and environmental factors (Thies et al., 1991;

Hardarson, 1993). Truchet and Dazzo (1982) observed that the addition of at least 18 mM of nitrate to the roots of alfalfa seedlings completely inhibited accumulation of *R. meliloti* cells on root hairs, root hair curling, infection thread development, and nodule formation, suggesting that nitrate may influence the signal-response between the two partners. Other studies also suggest that combined N alters nodule occupancy of strains of soybean *Rhizobium* (McNeil, 1982).

Thies et al. (1991) reported that, in the absence of indigenous rhizobia, the response to inoculation is directly proportional to the level of available soil N. A few reports (Bergensen et al., 1989; Brockwell et al., 1989) indicate that high rates of inoculation can improve inoculation response in the presence of high nitrate. Working with a high nitrate soil, Herridge et al. (1984) observed that increasing the rate of inoculum resulted in higher soil numbers of rhizobia in the rhizophere, improved nodulation and N_2 fixation, and a larger residual population of rhizobia the following year. The explanation advanced for these observations was that concentrations of nitrate in the soil water were not uniform and that the parts of the root system exposed to low concentrations of nitrate were nodulated. However, these conditions would most likely be satisfied when large populations of rhizobia were extensively distributed through the soil by applying heavy rates of inoculant. Spraying a water suspension of *B. japonicum* strain CB1809 directly into the seed bed (containing extractable mineral N from 37.6 to 18.5 mg N per kg dry soil) at 100 times the normal rate, resulted in significant colonization of the seedling rhizosphere by rhizobia and significant nodulation (Brockwell et al., 1989). Similar results were reported by Bergensen et al. (1989). Even though it is not economical to inoculate legume crops at such a high rate, this illustrates that the detrimental effect of combined N on nodulation and N_2 fixation can be ameliorated by proper inoculation strategies.

2.3.5 Effect of indigenous rhizobial population

In most bacteria, including rhizobia, the ability to establish and maintain themselves in the soil depends on their ability to compete with the indigenous population (Hicks and Loynachan, 1989; Thies et al., 1991; Toro, 1996). Where naturalized rhizobia are few or absent, the introduction of a new strain by inoculation of seed or soil is normally successful, provided other factors are favourable (Brockwell et al., 1995). In their investigations, George et al. (1987) and Abaidoo et al. (1990) concluded that in the absence of indigenous rhizobia, nodulation is a stable characteristic of the introduced rhizobial strains as long as plant growth conditions are favourable. On the other hand, where large populations of indigenous rhizobia occur, competition for nodule occupancy becomes a major factor determining the crop response to inoculation (Dowling and Broughton, 1986; Thies et al., 1991; Bottomley, 1992; Keyser and Li, 1992; Thies et al., 1992; Brockwell et al., 1995).

Indigenous rhizobia often occur in high numbers and are well adapted, giving them an advantage in certain aspects of competition, such as bacterial motility, attachment and nodule initiation (Keyser and Li, 1992; Thies et al., 1992). Consequently, indigenous strains dominate the nodules, and response to inoculation is usually not observed (Kapusta and Rouwenhorst, 1973; Kvien et al., 1981; Ge and Xu, 1982). For example, Ireland and Vincent (1968) observed that an inoculant, supplying 10^3 rhizobia seed^{-1}, was inadequate to nodulate white clover (*Trifolium repens*) when the introduced strain was outnumbered by clover rhizobia already present in the soil. In such situations, the application of massive inoculant rates can overcome the competition from indigenous rhizobia (Kapusta and Rouwenhorst, 1973), but such a delivery system would be more practical with soil inoculation.

2.3.5.1 Relationship between inoculum rate and nodule occupancy

Increased inoculum rates enhance the competitive advantage of rhizobia introduced into soil, although a threshold value typically occurs above which additional inoculum did not increase the competitive success of the isolate (Ireland and Vincent, 1968; Hiltbold et al., 1980; Brockwell et al., 1982; Singleton and Tavares,

1986). Increasing inoculum rate within the range of $\log_{10}$ 0.32 to 6.28 per seed in 7 and 10-fold increments improved colonization of lupin rhizospheres and increased nodulation (Roughley et al., 1993). They observed that, when the seed was inoculated with either $\log_{10}$ 6.27 or 5.27 bradyrhizobia per seed, more than 90% of the plants were nodulated after 43 d compared to 12, 21 and 34% for plants inoculated with $\log_{10}$ 1.27, 2.27 and 3.27, respectively.

Caldwell and Vest (1970) reported that the nodule occupancy of introduced rhizobia averaged 0.5 to 10% in soil with an established indigenous population. Others, however, have reported that nodule occupancy by introduced rhizobia can be increased, on the average, to 20% by increasing the inoculum rate (Kuykendall and Weber, 1978). Johnston et al. (1965) increased the proportion of inoculum-produced nodules from 5% with the standard rate of inoculum to as high as 25% with a rate 25 times the standard rate.

In a field trial, Weaver and Frederick (1974b) demonstrated that to achieve nodule occupancy greater than 50% in soybean, the bradyrhizobial number must be at least 1,000 times greater than the estimated number of indigenous rhizobia. Similar results were obtained in soybean by Pinochet et al. (1993) with *B. japonicum* in French soils. Recent field inoculation trials at five ecologically diverse sites, using several legumes, revealed that in the presence of an indigenous rhizobial population, the population of seed-applied *B. japonicum* must be 70 times that of the indigenous population to occupy $\leq$ 15% of the soybean nodules (Thies et al., 1992).

Brockwell et al. (1987) used three closely related strains of *B. japonicum* to inoculate each of three successive crops of soybean grown at the same site to evaluate the population dynamics of these strains. They found that in soil initially free of *B. japonicum*, rhizobial populations around the young seedlings were related to inoculum rates. Although nodule occupancies for the second and third years were dominated by naturalized *B. japonicum* strains, the magnitude of domination was reduced by increased rates of inoculum.

Many models, relating nodule occupancy to the numbers of indigenous rhizobia and the number of rhizobia applied as inoculant, have been proposed (Bohlool

and Schmidt, 1973; Marques Pinto et al., 1974; Amarger and Lobreau, 1982; Thies et al., 1991). Bohlool and Schmidt (1973) observed that the percentage of nodules formed by a particular rhizobial strain varied proportionally with the logarithm of the number of rhizobia in the inoculum. For *Rhizobium leguminosarium* bv. *phaseoli*, Beattie et al. (1989) presented a model in which a linear relationship between the logarithm of the nodule occupancy by the inoculant strain (A) and the logarithm of the ratio of inoculant strain (A) to the indigenous rhizobia (S) is described by the following equation:

$$\text{Log}[P_A/(1-P_A)] = CI_{A:S} + k\log[I_A/I_S] \qquad [2.1]$$

where, P_A the proportion of nodules occupied by strain A; I_A is the number of rhizobial cells applied to the seed; I_S is the number of indigenous *R. leguminosarium* bv. *phaseoli* cells per gram soil; $CI_{A:S}$ is the intercept, i.e., the competitive index (a positive value indicates A is more competitive than S); and k is the slope. Similar models have been developed to assess and compare the competitiveness and nodulation success of *R. leguminosarium* and *R. meliloti* (Marques Pinto et al., 1974), various strains of *R. leguminosarium* bv. *trifolii* (Labandera and Vincent, 1975) and various rhizobial strains for faba bean and alfalfa (Amarger and Lobreau, 1982).

In an extensive study at several locations in Hawaii, using various levels of available soil N and indigenous rhizobial populations, Thies et al. (1991) observed that inoculation responses were inversely related to the number of indigenous rhizobia. They developed the following equation describing the hyperbolic relationship between the yield response to inoculation and the size of the indigenous rhizobia population (determined by most probable number (MPN) plant infection assay):

$$Y = (314.7 - 5.09 \times N_{min}) \times (1 + \text{number of indigenous rhizobia}) \qquad [2.2]$$

where Y is the percentage increase in yield due to inoculation and N_{min} is N mineralization potential ($\mu g\ N\ g^{-1}$ soil $week^{-1}$). The study demonstrated that the numbers of indigenous rhizobia accounted for 59% of the observed variation in

inoculation response, indicating that the size of soil rhizobial populations had a strong influence on the success of inoculation.

2.3.5.2 Effect of repeated inoculation on nodule occupancy

The intense competition from the indigenous population of rhizobia has made it difficult to establish introduced rhizobia strains in most soils. Most research on altering nodule occupancy is, therefore, directed at facilitating an immediate shift in strain distribution (Miller and May, 1991). As an ideal, producers would prefer to forgo inoculating every time they grow the same legume crop on the same field. However, this view is probably not shared by many *Rhizobium* researchers and inoculant companies as better performing *Rhizobium* strains are being identified or constructed by various methods (Evans et al., 1987; Paau, 1989; Bosworth et al., 1994; Sharypova et al., 1994).

Nevertheless, some rhizobial strains introduced to the soil can persist for many years and many compete directly with subsequent inoculant rhizobia for nodulation (Kamicker and Brill, 1987). Dunigan et al. (1984) reported that repeated massive inoculation with a competitive strain eventually changed nodule occupancy in soil containing 3×10^5 indigenous rhizobial cells g^{-1} soil. In this seven-year study, *B. japonicum* strain USDA 110 was used as the soil inoculum at 1×10^8 cells per cm row for three successive years. The recovery of strain USDA 110 in soybean nodules was approximately 4, 6, and 7% in the first three years, respectively. However, recovery for the fourth year reached 17%, and 54% by the seventh year. McLoughlin et al. (1990a) examined the establishment and persistence of six introduced *B. japonicum* strains over three years in Wisconsin soil with a low indigenous population of *B. japonicum* (≤ 10 rhizobial cells g^{-1} soil). In their study, application of liquid inoculum at a high rate of 1×10^8 rhizobial cells per 2.5 cm row to the seed furrow produced 100% nodule occupancy in the first growing season. Without further inoculation in the second and third year, they found that 60% of the nodules from all plots was formed by the introduced strains.

In spite of the many successes achieved in increasing the nodule occupancy by inoculant strains with high doses of inoculum, massive inoculation does not always enhance nodule occupancy. For example, the nodule occupancy of *R. leguminosarum* bv. *trifolii* strain 285 was not related to the inoculum concentration, but to the high competitive ability of the strain (Martensson, 1990). In a similar manner, Kamicker and Brill (1987) reported that, in addition to increased inoculum rate, inoculum placement also influenced nodule occupancy.

2.3.5.3 Inoculum placement and nodule occupancy

Rhizobia move through the soil either actively with their flagella or passively by water movement (Issa et al., 1993a,b). Rhizobial movement, however, is possible only when the soil is saturated or at a nearly saturated water capacity (Vlassak and Vanderleyden, 1997). Bacterial movement is restricted below field capacity, since larger pores are filled with air and soil water occurs as a discontinuous film, (Chamblee and Warren, 1990; Worrall and Roughley, 1991). Madsen and Alexander (1982) reported that *B. japonicum* did not move beyond 2.7 cm in the absence of percolating water. Consequently, it has been argued that a method of inoculation that provides a greater spatial distribution of introduced rhizobia would increase the chances of the inoculum coming into contact with the emerging root hairs of the host plant (Date, 1991; Brockwell et al., 1995).

Seed inoculation, either by peat or liquid inoculant, often results in a high density of rhizobial cells near the seed with nodulation restricted to the upper tap root (Worrall and Roughley, 1976; Danso and Bowen, 1989; Hardarson et al., 1989; Danso et al., 1990; Ciafardini and Lombardo, 1991). Nodulation of the more distal parts of the tap root and the lateral roots by the inoculant strain is reduced, due to the low density of this strain in the vast bulk of the soil (Weaver and Frederick, 1974a,b; Wadisirisuk et al., 1989). Kamicker and Brill (1987) evaluated the ability of three strains of *B. japonicum* to form nodules on field-grown soybean in soil with a highly competitive indigenous *B. japonicum* population. They observed that increasing inoculum rates resulted in a higher proportion of the nodules being formed by the

introduced inoculant strain. Moreover, the vertical distribution of the nodules, containing the inoculant strain, was affected by the method of adding the inoculant to the soil. In their study, a larger proportion of nodules, containing the inoculant strains, was formed in the lower part of the root when the inoculant was tilled into the soil as compared to when the same amount of inoculant was added to the seed furrow only. They concluded that at least 10^9 rhizobial cells must be added to each seed and surrounding soil to form at least 50% of the nodules when the indigenous population was 10^3 cells g^{-1} soil. Rice and Olsen (1992) similarly observed that, on a moderately acid soil, granular inoculant applied with or below the seed resulted in greater nodule occupancy than when applied in the seed row. In addition, granular inoculant applied with or below the seed was more effective at a site with a population of low indigenous *R. meliloti* than at a site with a higher population.

Competition for nodule occupancy is a complex phenomenon with interactions among the bacteria, the host and the environment. However, the above findings clearly indicate that the best way to establish a new strain of rhizobia within a naturally occurring population is to apply a heavy rate of effective, persistent inoculum strategically close to the growing legume roots. Such an inoculant delivery system is practical with soil inoculation, but the accuracy of the placement could be improved and the concentration increased by using seeding equipment with attachments that place the granular inoculant in the seed bed or below the seed (Muldoon et al., 1980; Brockwell et al., 1987).

2.4 Effect of inoculation method on nodule formation and activity, and yield

Methods of rhizobial inoculation can have a great influence on the extent of nodulation (Smith and del Roi Escurra, 1982; Rice and Olsen, 1988; Danso et al., 1990), nodulation pattern, the amount of N_2 fixed (Kamicker and Brill, 1987; Hardarson et al., 1989; McDermott and Graham, 1989; Ciafardini and Lombardo, 1991) and yield (Bezdicek et al., 1978; Muldoon et al., 1980). Increased inoculum rates result in increased nodulation and N_2 fixation, especially under stress conditions. Moreover, the depth of inoculum placement in the soil can affect the location of the

nodules on the root system (Wadisirisuk et al., 1989), subsequently influencing the onset of nodule activity and the amount of N_2 fixed over the entire growing season (Wadisirisuk et al., 1989; Hardarson, 1993).

2.4.1 Nodulation and nodulation pattern

The location of nodules on the roots depends to a large extent on the inoculation procedure, timing of application and depth of inoculum placement (Ciafardini and Barbieri, 1987; Kamicker and Brill, 1987; Danso and Bowen, 1989; Hardarson et al., 1989; McDermott and Graham, 1989; Wadisirisuk et al., 1989; Danso et al., 1990; Ciafardini and Lombardo, 1991; Ocumpaugh and Smith, 1991). Nodule formation is restricted to the vicinity of inoculum placement due to the limited movement of rhizobia in the soil and rhizosphere. Thus, with seed inoculation, most of the nodules occur at the crown region of the roots, whereas soil inoculation, particularly below the seed, results in the formation of nodules on the lower portion of the roots. For this reason, Zablotowicz et al. (1991) suggested that more uniform dispersion of inoculum would be desirable, but this would require the addition of higher levels of inoculum to the soil. Caetano-Anolles et al. (1992), working on growth and movement of spot-inoculated *R. meliloti*, concluded that the rate of movement and multiplication of rhizobia did not occur fast enough to keep up with the rate of root elongation. They observed that most of the nodules developed near the inoculation site, with more nodules at higher inoculum rates.

Wilson (1975) placed a liquid suspension of rhizobial cells at 1.5, 10 and 20 cm below the surface of soil in pots in the greenhouse to evaluate the influence of inoculum placement on the nodulation pattern. He found that 84 and 83% of the nodules from the 10 and 20 cm inoculation, respectively, occurred deeper than 7.5 cm below the soil surface, but only 15% of the nodules from the 1.5 cm inoculation was formed deeper than 7.5 cm below the soil surface.

Using *B. japonicum* strains 110 and 142 separately in peat, and in granular formulations, Bezdicek et al. (1978) reported that granular inoculum enhanced nodulation by strains 110 and 142 by 14 and 19%, respectively, over seed treatment

with peat inoculant. They also observed that doubling the granular inoculum rate significantly increased nodulation. In groundnut (*Arachis hypogaea*), soil-applied inoculum produced 41.8 nodules per plant with nodule dry weight of 3.92 mg, whereas seed-applied inoculum resulted in 25.5 nodules per plant with nodule dry weight of 2.77 mg (Hedge and Brahmaprakash, 1992). Soil inoculation produced more than four times the number of nodules with about twice the dry weight on soybean roots compared to that for seed-applied inoculant (Muldoon et al., 1980).

Using a rhizobial suspension for soil inoculation on soybean, Danso and Bowen (1989) observed that soil inoculation produced over 50% more nodules than seed-applied inoculation, although nodule weight was similar. They also found that seed inoculation produced 94% of the nodules at 0-5 cm from the stem base compared to 63% with soil inoculation. Similar results were reported subsequently by Danso et al. (1990).

In a greenhouse study, inoculation of soybean seed resulted in fewer nodules and the nodules were located predominantly on the tap and crown roots within 0-5 cm from the stem base as compared to treatments where the bradyrhizobia were distributed throughout the soil or placed at specific depths (Wadisirisuk et al., 1989). In general, they observed maximum nodulation at the 5-cm zone immediately below the level at which the inoculum was placed. For instance, for the 5 and 10-cm placement, this zone developed 56 and 53% of the nodules, respectively, 75 days after planting. Similarly, Ocumpaugh and Smith (1991) examined early- and late-planted arrowleaf clover (*Trifolium vesiculosum*) in the field and observed that when granular inoculum was placed with untreated seed at planting, nodulation of tap and lateral roots was superior to the seed-inoculated treatments. In greenhouse and field studies, Hardarson et al. (1989) used different inoculation techniques, including peat-based seed inoculation, soil inoculation by mixing *Bradyrhizobium* with soil, inoculum placed at the level of seeding and inoculum placed 5 cm below the seed. They reported that seed inoculation produced most of the nodules on the crown of the roots, in contrast to the profuse and well-distributed nodules when the inoculum was applied throughout the soil. Furthermore, most nodules were produced in the lower portions of

the root when the inoculum was placed below the seed. In a similar study, Kamicker and Brill (1987) also found that inoculant added to the seed furrow produced nodules mainly in the top portion of the soybean root system, whereas inoculant incorporated into the soil produced nodules mostly in the lower portion of the root system.

As a result of the enhanced nodulation with soil inoculation, Brockwell (1985) argued that inoculant, placed in the seed zone of the soil, is relatively far from the infectible region of the seedling roots. Moreover, this situation is compounded by the limited mobility of the inoculant rhizobia. This is one of several reasons that justifies the use of alternative inoculation methods, such as the use of granular or liquid inoculant applied uniformly to the seed bed.

2.4.2 N_2 fixation

Although estimates of N_2 fixation in both greenhouse and field conditions are variable, soil inoculation usually results in enhanced N_2 fixation as compared to seed-applied inoculant, particularly under unfavourable soils conditions (Scudder, 1975; Hardarson et al., 1989; Danso et al., 1990). Wadisirisuk et al. (1989), using an ^{15}N-isotope-dilution method, showed that mixing inoculum with the soil or placement below the seed resulted in greater N_2 fixation both in terms of the percentage and total N fixed at 55 and 75 days after planting. In Ontario, the amount of N_2 fixed, as estimated by acetylene reduction and averaged over three locations, was 94% greater for granular (soil-applied) inoculant as compared to seed inoculation (Muldoon et al., 1980). Methods of inoculation greatly influence the proportion or amount of N_2 fixed by legumes through the effects on nodulation patterns (Danso and Bowen, 1989; Wadisirisuk et al., 1989), and the onset and duration (Zapata et al., 1987; Imsande, 1989) of N_2 fixation.

2.4.2.1 Crown vs. lateral root nodules

While nodules at the crown region are active during the early stage of plant growth, N_2 fixation declines early in the growing season. For example, Bergensen (1958) reported that N_2 fixation in soybean declined significantly by 65 days after planting. Nodules on the lower root system and lateral roots are formed later and continue fixing N_2 longer (Ciafardini and Barbieri, 1987; Hardarson, 1993). Therefore, nodulation on the lower part of the root system may be essential for maximum N_2 fixation, in order to match the high N demand during pod fill (Imsande, 1989).

In soybean, McDermott and Graham (1989) demonstrated that crown root nodules accounted for 100% of the acetylene reduction activity at 20 days after planting, but the contribution declined to about 20% at 76 days after planting. Greenhouse and field experiments in another study also showed that the position of the nodules on the root system of soybean had a greater influence on the amount of N_2 fixed than the number or fresh weight of nodules (Hardarson et al., 1989). In the greenhouse, N_2 fixation was estimated by an ^{15}N-isotope-dilution method. Results indicated that all of the treatments in which the bradyrhizobia were inoculated into the soil, and which had most of the nodules formed at the 5 to 15 cm soil depth, derived more than 90% of their N from the atmosphere. In contrast, plants inoculated with a seed-applied inoculant had greater total nodule dry weight with most of the nodules in the top 5 cm of the root system, but derived only 15% of their N from the atmosphere. Although the response in the field was not as high as observed in the greenhouse, the trend was similar (Hardarson et al., 1989).

Wolyn et al. (1989), using the non-quantitative acetylene reduction technique, similarly reported higher acetylene reduction values for common bean nodules on lateral roots at all growth stages beyond R3 (50% bloom) compared to that of the crown-root nodules, even though average nodule weight did not differ at any stage. In addition, they found that the leghemoglobin concentration in the lateral-root nodules was greater than that in the crown-root nodules after the R3 stage. At the late pod-filling stage, lateral-root nodulation scores correlated positively with acetylene reduction and leghemoglobin content ($r = 0.72$ and $r = 0.66$, respectively), whereas no

correlation was detected for crown-root nodulation scores. In a field study with common bean, Vikman and Vessey (1992) also reported a sharp decline in acetylene reduction rates of the crown-root nodules of bean with the onset of pod filling in contrast with that of the non-crown-root nodules. The acetylene reduction rates for the non-crown-root nodules was maintained through the pod-filling stage and was four times higher than that of the crown-root nodules around the mid pod-filling stage. In another study, the authors observed a sharp drop in nitrogenase activity in the nodules on the top part of the root system to a third of its previous level at 63 days after planting, whereas that of the nodules on the mid part of the root system remained unchanged or increased (Vikman and Vessey, 1993). Apparently the lack of inoculum at the distal parts of legume roots resulted in a decline in N_2 fixation at the onset of pod filling. Thus, a method of inoculation that delivers *Rhizobium* to the lower portions of the root system should enhance the proportion or amount of N_2 fixed.

2.4.2.2 Time course of nodule activity

The amount of N_2 fixed is affected by the length of time a legume actively supports N_2 fixation (Hardy, 1977), which, in turn, is influenced by inoculation method and the depth of placement of the inoculum. Nitrogen fixation generally reaches a peak at the early pod-filling stage and declines during the late reproductive phases (Latimore et al., 1977; Imsande, 1989). Pena-Cabriales et al. (1993) found that N_2 fixation in common bean, as estimated by ^{15}N isotope dilution, increased up to 63 and 77 days after planting for greenhouse and field-grown plants, respectively, and thereafter declined. Assessment of nitrogenase activity, using acetylene reduction assays, also indicted that the activity increased until the reproductive stages and then decreased to undetectable levels during the late pod-filling stage (Pena-Cabriales et al., 1993). As determined by the A-value method, the maximum rate of N_2 fixation for soybean was observed between the R1 and R3 growth stages (pod fill), after which the amount declined by half between the R5 and R7 growth stages (between pod fill and physiological maturity) (Zapata et al., 1987).

Kumaga et al. (1994) found that N_2 fixation in bambara groundnut (*Vigna subterranea*) reached its peak at the mid pod-filling stage; thereafter, N_2 fixation by cv. Ex-Ada declined to an undetectable level, whereas cv. CS-88-11 maintained N_2 fixation up to physiological maturity. This cultivar difference may be due to the differences in growth habit, since Ex-Ada is a bunch type, whereas CS-88-11 is a slightly spreading type that matures two weeks later than Ex-Ada. In a growth chamber study, Vessey (1992) found that N_2 fixation, as estimated by nitrogenase activity, declined in field pea with the onset of pod filling in the determinate cultivar Express, whereas N_2 fixation in the indeterminate cultivar Century did not reach its peak until several weeks into the pod-filling stage. However, under field conditions, N_2 fixation dropped sharply with the onset of pod filling in Century. This decline was attributed, in part, to environmental conditions, e.g., water stress. Graham and Rosas (1977) and Rennie and Kemp (1983) also showed that indeterminate cultivars of common bean fixed more N over the growing season than determinate cultivars.

Although maintenance of N_2 fixation into the pod-filling period is dependent on genetic and environmental factors, it should be possible to enhance N_2 fixation by inducing optimum nodulation on the lateral roots. In all the studies discussed above, seed inoculation methods were used and it is likely that almost all of the nodules were formed at the crown region or top part of the root system. These nodules enter into a stage of senescence at relatively early plant growth stages (Bergensen, 1958), and are also in the layer of soil that is subject to great fluctuation in both temperature and moisture (Wilson, 1975) with the onset of pod filling. Thus, it is likely that N_2 fixation could be enhanced by a method of inoculation that provides deeper placement of inoculum in the soil to minimize adverse environmental effects on nodules and also inoculates more of the root system, instead of only the crown. Several studies have shown that lateral-root nodules are responsible for maintaining or even increasing nitrogenase activity during the pod-filling stage (Wolyn et al., 1989; Vikman and Vessey, 1992, 1993).

2.4.3 Yield and quality

Considerable yield increases have been reported in several studies with granular inoculants, particularly under adverse environmental conditions. Scudder (1975) obtained yield increases in soybean of up to 38% for granular-applied inoculant over seed-applied inoculant under hot and dry conditions in Florida on a field that had not been previously cropped to soybean. In contrast to this observation, Nelson et al. (1978) reported that yield and total N content in the leaves and grain of soybean were not affected by either granular or seed-applied inoculants. This suggests that the soils had an adequate population of rhizobia for nodulation and indicates that routine inoculation of soybean may not be necessary when soybean is grown frequently.

In Ontario, granular inoculant increased soybean seed yields by 20% over seed-applied treatments and 48% over the non-inoculated control in a two-year study (Muldoon et al., 1980). The authors further found that soil-applied inoculants increased seed protein content by 7%, while oil content decreased by 3%. Brockwell et al. (1980) evaluated methods of inoculation with several legumes including chickpea, soybean and field pea. They concluded that soil inoculation was superior to seed inoculation in foliage dry weight when seeds were treated with fungicide. However, when fungicide was not used, responses to inoculation generally were equally good for all three forms of inoculation (granular, liquid, seed applied). They also demonstrated that increasing the rate of soil inoculation, which may not be practical with seed inoculation, often resulted in higher grain yield. Bezdicek et al. (1978) also reported a yield advantage for granular-applied inoculant over seed-applied inoculant with the same strain. In the study by Bezdicek's group, the yield for the soil-applied inoculant was 60% higher than for the seed-applied inoculant.

High yield in soybean has been reported with the use of granular inoculant, even when 160 kg ha^{-1} N was applied (Dubetz et al., 1983). This indicates that the granular inoculum not only fixed enough N for optimum yield, but the ^{15}N data (Rennie et al., 1982) also showed that the soybean in a fixing mode apparently ignored the applied fertilizer N (Dubetz et al., 1983). Chamber (1983) examined the influence of several methods for rhizobial inoculation on nodulation and yield of soybean in

Spain. He found that, compared to seed inoculation, inoculating the furrow with solid inoculum gave good plant growth, which correlated positively with grain yield and protein concentration. In field trials, using faba bean (*Vicia faba* L.) in several locations in Manitoba, Dean and Clark (1977) observed that granular inoculum increased plant vigor from an early stage relative to seed-applied inoculum. They reported that when soil moisture was low, granular inoculant resulted in a yield enhancement of 730 kg ha^{-1} compared to seed-applied inoculant.

Granular inoculants increased yield of lentil (*Lens culinaris*) in small plots and on-farm field trials by 16% and 36%, respectively, over seed inoculation (Stephens and Chamberlain, 1996). They also reported that granular inoculants provided a yield advantage of 13% above that of seed-applied inoculants for field pea over the period of 1991 to 1995.

Soil inoculation also increased yield of alfalfa on moderately acid soils (Rice and Olsen, 1988). In another study, Rice and Olsen (1992) compared soil-applied inoculants with an uninoculated control and the conventional seed-applied inoculants. In this experiment, using alfalfa on a moderately acid soil (pH 5.8), it was concluded that granular inoculant applied with or below the seed resulted in a significant yield increase over the conventional seed-applied inoculant at a site with a normal indigenous population. In arrowleaf clover, Ocumpaugh and Smith (1991) found that granular inoculant with the seed resulted in more vigorous seedlings with nearly double the dry matter yield of those with the seed-applied inoculant.

It can be argued at this point that the importance of delivering large numbers of rhizobia is a challenge, and the best system to date is the soil-applied granular inoculants. Brockwell et al. (1995) in a recent review concluded that soil inoculation is often better and never worse than conventional seed inoculation for initiating nodulation and N_2 fixation. Soil inoculation facilitates the application of large numbers rhizobia for more effective nodulation and N_2 fixation, while providing a micro-habitat that helps protect the rhizobia from harsh environmental conditions. If legumes are cultivated on soils with low available soil moisture, high temperature, low acidity or other forms of adverse environmental conditions that affect the viability of the

introduced rhizobia, then the use of granular inoculant may be the best agronomic practice.

3. EFFECT OF INOCULUM PLACEMENT ON NODULATION AND N_2 FIXATION BY CHICKPEA

3.1 Introduction

Chickpea can obtain a significant portion of its N requirement through symbiotic N_2 fixation when grown in association with effective and compatible *Rhizobium ciceri* strains (Beck et al., 1991; Beck 1992). The crop is new to Saskatchewan, and, because the soils do not contain sufficient numbers of the specific rhizobia if present (Rennie et al., 1982; Hynes et al., 1995), inoculation is necessary to provide sufficient numbers of the correct rhizobial strain for effective nodulation and N_2 fixation. However, the success of inoculation often is limited by several factors, including environmental conditions (Bottomley, 1992; Graham, 1992), the number of infectious cells applied (Bissonnette and Lalande, 1988; Brockwell et al., 1995), the presence of competing strains of rhizobia (Thies et al., 1991, 1992) and the inoculation methods (Brockwell and Bottomley, 1995; Toro, 1996).

Several studies have shown that a large majority of the rhizobia, applied to seed via conventional seed inoculation, die on the seed prior to seeding or shortly after placement in the soil due to exposure to seed treatment chemicals, seed coat toxins, dehydration or excessive heat (Brockwell et al., 1980; Roughley et al., 1993). Consequently, a method of inoculation in which the inoculum can be applied directly to the soil in high doses, and at the same time remain protected from adverse environmental conditions, has received much attention (Wilson, 1975; Bezdicek et al., 1978).

Scudder (1975), using granular inoculant in the seed furrow, obtained a 38% yield increase over seed-applied inoculant in soybean under hot and dry conditions in Florida. Similarly, Bezdicek et al. (1978), working with soybean, found that placing granular inoculant in the soil with the seed was superior to seed-applied inoculant. Brockwell et al. (1980) summarized the results of experiments with several legumes,

including chickpea, where granular inoculant was used. They found that, when conditions were unfavourable for the survival of rhizobia, or when germination was delayed due to environmental conditions, soil inoculation resulted in better nodulation and often better plant growth and yield than seed-applied inoculants. Other investigators working with soybean (Muldoon et al., 1980; Chamber, 1983), faba bean (Dean and Clark, 1977), arrowleaf clover (Ocumpaugh and Smith, 1991) and alfalfa (Rice and Oslen, 1988, 1992) have reported similar findings.

The depth of inoculum placement is an important factor that can influence the benefits of granular inoculation. It is well established that movement of rhizobia in the soil is limited (Madsen and Alexander, 1982; Kamicker and Brill, 1987). This finding is supported by reports that seed-applied inoculum or granular inoculum at the seeding depth results in nodulation predominantly in the crown region of the root system (Danso and Bowen, 1989; Hardarson et al., 1989; Danso et al., 1990). Contrary to the belief that crown-root nodules are of supreme importance, McDermott and Graham (1989), Wolyn et al. (1989) and Vikman and Vessey (1992), using the non-quantitative acetylene reduction assay, have shown that lateral-root nodules which were formed later are more active during pod filling and seed maturation and can provide significant fixed N during later reproductive stages of the plant as compared to crown nodules. Thus, inoculation strategies, aimed at positioning the inoculant rhizobia to intercept lateral roots, can improve nodulation of the lower part of the root system and, consequently, improve fixation. Hardarson et al. (1989) and Wadisirisuk et al. (1989) demonstrated this in soybean by placing the inoculum below the seed. However, none of the studies examined the optimum placement depth for effective nodulation and N_2 fixation. Therefore, the objectives of this study were to: 1) evaluate the effect of seed and soil inoculation methods on nodulation, N_2 fixation and yield of chickpea; 2) determine the optimum placement depth for granular inoculum; and 3) examine the contribution of lateral-root nodules to N_2 fixation and yield.

3.2 Materials and methods

3.2.1 Study sites and soil test

In 1997, field experiments were conducted at four sites in Saskatchewan: near Elbow, Kenaston, Outlook and Watrous. Another site on the same farm near Outlook, as well as a site on the same farm near Watrous, were used for similar studies in 1998. The sites were located in the Dark Brown soil zone and were within commercial fields. The soils were classified as Orthic Dark Brown Chernozems, according to the Canadian System of Soil Classification (Soil Classification Working Group, 1998). These sites were selected because of low soil N levels and the absence of a history of chickpea production. Soil sampling was carried out prior to seeding at each location in the spring of 1997 and 1998. Chemical analyses of soil samples for pH and conductivity (determined on a 1:1 soil:water suspension (Hogg and Henry, 1984)); NO_3-N (calcium chloride extractable); P and K (sodium bicarbonate extractable (Olsen et al., 1954)) were performed by Enviro-Test Laboratories, Saskatoon, SK (Table 3.1). Soil moisture content was also determined. The soil at Kenaston was also sampled in the fall of 1997, but because the results were similar to those obtained in the spring, data are not presented. Chickpea was grown on samples of the soils obtained from each site (0-30 cm depth), but did not nodulate after six weeks in a pot experiment in a growth chamber, confirming the absence of *R. ciceri*.

3.2.2 Experimental procedure

A randomized complete block design with four replications was used at all sites. Each experiment consisted of 11 inoculation treatments with either desi (cv. Myles) or kabuli (cv. Sanford) chickpea (*Cicer arietinum* L.). In 1997, the desi chickpea was planted on May 14 at Elbow, Kenaston and Outlook, and on May 20 at Watrous. For the 1998 desi experiments, planting was on May 9 and 20 at Watrous and Outlook, respectively. Each plot was planted with a double disc press drill with separate discs for seed and fertilizer placement (Fabro Ltd., Swift Current, SK) and consisted of 7 rows (six chickpea rows and one flax row) 12 m long and 15 cm apart. Duplicate experiments with kabuli chickpea were conducted at both Kenaston and Watrous in 1997 and at both Outlook and Watrous in 1998. The seeding rate for desi

chickpea was 110 kg ha^{-1} and 160 kg ha^{-1} for kabuli chickpea (Saskatchewan Pulse Crop Development Board, 1997).

Table 3.1. Soil test data (0-30 cm) from the experimental sites prior to seeding, 1997 and 1998.

Locations	Gravimetric moisture content (%)	pH	E C (mS cm^{-1})†	NO_3-N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})
			1997			
Elbow	9.5	7.9	0.33	8.8	11.2	440
Kenaston	13.1	8.2	0.45	8.4	6.6	240
Outlook	16.4	7.1	0.82	10.8	9.2	540
Watrous	19.4	7.5	0.48	9.2	32.0	540
			1998			
Outlook	10.6	8.3	0.5	12.4	18.4	440
Watrous	19.4	8.1	0.2	16.4	12.4	540

†EC values < 2 indicate that salinity effects are usually negligible (Bower and Wilcox, 1965).

Six commercial inoculants of *Rhizobium ciceri* (Table 3.2) were applied each year at the recommended rate. Eleven inoculation treatments were used: 1) seed inoculation using two different peat inoculants (A or B brand) or two different liquid inoculants (A or B brand); 2) soil inoculation, with two granular inoculants (A or B) placed either in the furrow with the seed at planting, side banded 2.5 cm below the seed or 8 cm below the seed and 3) a non-inoculated control. Inoculants with the same designation, e.g., A, indicate that the identical *Rhizobium* strain or strains were used in the different carriers. Inoculant A contained a single strain, CP39 (ICARDA, Aleppo, Syria; and kindly formulated by MicroBio RhizoGen Corp., Saskatoon), whereas inoculant B contained a mixture of three strains, 27A2, 27A7 and 27A9 (LiphaTec Inc., Milwaukee, WI). The liquid formulation of inoculant B was not available in

1997; hence, an experimental liquid formulation (Inoculant C), containing single strain 27A2 (Agrium Biologicals Inc., Saskatoon, SK), was used.

Seed inoculation was performed by throughly mixing a measured amount of peat or liquid inoculant according to the manufacturer's recommendation, with 1.5 kg seed and using 5 ml of 1% gum arabic solution as sticker in plastic bags immediately before seeding. The granular inoculants were soil-applied either in the seed row or to the side of the seed row at different depths, using a second set of discs (adjusted for the various depths). Triple superphosphate (0-45-0) was applied at planting in the seed row at the rate of 20 kg ha^{-1} P_2O_5. To minimize contamination, the non-inoculated plots were planted first. In addition, all treatments with the same rhizobia strain(s) were planted consecutively before switching to other treatments to minimize the potential for inadvertently contaminating the treatments. Moreover, the planter was thoroughly cleaned with a vacuum cleaner and then disinfected with 70% ethanol after planting each treatment plot. Flax was used as the reference crop for the assessment of percentage N derived from the atmosphere (%Ndfa).

Weeds were controlled by hand hoeing during the growing season. The plants relied on natural precipitation throughout the growing season. Mean monthly precipitation and mean maximum air temperature for the various sites for the 1997 and 1998 growing seasons are presented in Appendix 1.

Table 3.2. Name, designation, manufacturer and the rates of commercial inoculants used in 1997 and 1998.

Rhizobium strain	Designation	Manufacturer	Application rate
CP 39	Liquid A	MicroBio RhizoGen	4.5 ml/kg seed
27A2	Liquid C[†]	Agrium Biologicals Inc.	4.5 ml/kg seed
27A2, 27A7, 27A9	Liquid B	LiphaTec Inc.	4.5 ml/kg seed
CP 39	Peat A	MicroBio RhizoGen	1.95 g/kg seed
27A2, 27A7, 27A9	Peat B	LiphaTec Inc.	6.15 g/kg seed
CP 39	Granular A	MicroBio RhizoGen	9.0 kg/ha
27A2, 27A7, 27A9	Granular B	LiphaTec Inc.	5.6 kg/ha

[†]Liquid formulation C was used in 1997 instead of Liquid B because it was not available.

3.2.3 Sample collection and analysis

In 1997, sampling was performed by randomly excavating the root systems of five plants to a depth of approximately 20 cm from the central rows of each plot at the flowering and early pod-filling stages for desi chickpea and at the early pod-filling and late pod-filling stages for kabuli chickpea. Soil adhering to the roots was carefully removed and the whole plants and dropped nodules were bagged and transported to the laboratory. Roots were gently washed under running tap water and nodules were collected. Nodules from the crown region and lateral roots were separated and counted. The crown region was defined as that part of the root extending 3 cm in all directions from the stem base, whereas the lateral roots were defined as that part of the root system extending beyond 3 cm from the stem base. The nodules and the whole plants were dried in an oven at 60 °C for 7 d and dry weights were determined.

At maturity, a 1-m^2 area of unsampled center rows of each plot was hand-harvested with a sickle. Whole plant samples were dried at 60°C for 48 h and subsequently weighed. Following biomass determination, the plants were threshed with a stationary thresher. Seeds were cleaned, weighed, and yields were calculated on a per hectare basis. The seed was milled to a < 2-mm particle size with a Wiley mill

(Arthur H. Thomas Company, Philadelphia, PA) and then finely ground by passing through a cyclone mill (Tecator model Cyclotec 1093) equipped with a 0.4-mm sieve. Seeds of flax were ground with a mortar and pestle. Approximately 1-mg samples of ground seed were analyzed for total N and atom percent ^{15}N excess with an isotope ratio mass spectrometer VG Micromass 602E (Isotech, Middlewich, England) (Bremer and van Kessel, 1990). Seed protein was determined by multiplying total N by the factor 6.25 (Tkachuk, 1969) and then expressed as protein concentration. Atom % ^{15}N excess was calculated with reference to the natural ^{15}N abundance of the atmosphere (0.3663 atom % ^{15}N) (Rennie and Kemp, 1984).

Data collection and analysis for the 1998 experiments were similar to the previous year except for the first sampling of the desi plots, which was done at the early pod-filling stage instead of the flowering stage. In addition, the plants were not sampled at the late pod-filling stage at the Outlook site since it was not possible to recover most of the nodules because the soil was too dry and difficult to excavate.

3.2.4 Calculations

Natural ^{15}N abundance was calculated according to Bremer and van Kessel (1990):

$$\delta^{15}N = \left[\frac{\text{atom \% } ^{15}N \text{ (sample)} - \text{atom \% } ^{15}N \text{ (standard)}}{\text{atom \% } ^{15}N \text{ (standard)}} \right] 1000 \qquad [3.1]$$

where the standard is atmospheric N_2 gas (0.3663 atom % ^{15}N).

The percent N derived from the atmosphere (%Ndfa) was then calculated as follows:

$$\%\text{Ndfa} = \left[\frac{(x-y)}{(x-c)} \right] 100 \qquad [3.2]$$

where x is $\delta^{15}N$ of seeds of plants deriving all their N from soil (in this case flax), y is the $\delta^{15}N$ in chickpea seed, and c is $\delta^{15}N$ of chickpea seeds from plants grown in an N-free medium (for details of the experiment see chapter 4). The c values for desi chickpea were 1.0009 and 1.0005 for the single strain CP39 and mixed strain (27A2, 27A7 and 27A9), respectively. The value for kabuli chickpea and rhizobial strain combinations was 1.0007.

3.2.5 Statistical analyses

Data for each site were analyzed separately, using the general linear model procedure of SAS (SAS Institute, 1996). The error terms for each year were examined for homogeneity of variance (Snedecor and Cochran, 1980), using Bartlett's test. For the 1998 data, Bartlett's test produced chi-squared values, which were not significant. Hence, the error terms for the Outlook and Watrous sites for each cultivar were considered homogeneous. Similarly, the error terms for all the parameters measured in the 1997 kabuli experiments were also homogeneous, according to Bartlett's test. On the other hand, some of the variances (e.g., for yield) for the 1997 desi experiments were heterogeneous. However, the variances were not too distinct from each other and, according to Gomez and Gomez (1984), if the highest error MS is not three-fold larger than the smallest error MS, the error variances can be considered homogeneous. Dr. R. J. Baker (personal communication) also argued that failure to correctly account for heterogeneous error variances would have little effect on the estimation of, or comparisons among, main effects of a fixed factor. In the analyses, inoculation treatment was considered a fixed factor; hence, heterogeneity of variances would not have much affect on the comparisons among treatment means. Therefore, combined analyses were conducted separately for the 1997 and 1998 experiments.

Significant differences among treatment means were evaluated with LSD at the 5% probability level. Orthogonal contrasts ($P \leq 0.05$) were used to statistically compare inoculant formulations and inoculation methods. The combined analysis of data over years was not performed because, although four similar experiments were conducted at four locations in 1997, only two experiments, each at one location were conducted for desi in 1998. For the kabuli, although two similar experiments were conducted in both years, one of the 1998 experiments was conducted at a site different from that of 1997. Moreover, liquid B was not available in 1997, so liquid C was used instead; therefore, one of the treatments was different between years. Correlation analyses of shoot dry matter per plant and seed yield ha^{-1} averaged over sites were performed separately on dry weight of crown nodules and lateral root nodules per plant averaged over sites.

3.3 Results

3.3.1 Individual plant data

3.3.1.1 Plant growth and nodulation

Moisture conditions at Watrous in both 1997 and 1998 favoured early seedling emergence, and plant growth was more vigorous than at the other sites (Appendix 1). However, plant growth at Elbow in 1997 was restricted by low soil moisture at seeding (Table 3.1), but this apparently did not affect plant growth response to inoculation. On the other hand, in 1998, seeding at Outlook was eleven days later than at Watrous due to drought conditions, but no rain occurred during this delay. The Outlook plots were seeded on 20 May and according to Environment Canada, average precipitation at Outlook for May 1998 was 57% less than normal (Appendix 1). As a result of the low soil moisture (Table 3.1), seedling emergence was slow and plant stand was low, particularly in treatments where granular inoculants were placed below the seed. The soil was very dry and it was observed that the upper 30 cm was very hard and difficult to penetrate with a shovel. The resistance encountered by the disc openers for both the granular inoculant (i.e. 2.5 and 8.0 cm below seed placement) and the seed prevented the discs from penetrating to the desired depth. Hence the seeds were deposited just below the soil surface where the soil moisture content apparently was too low for optimum germination, particularly for the large-seeded kabuli. Dry conditions during the later part of the growing season at Outlook in 1998 also made sampling for plant roots and attached nodules difficult and plans to sample roots at late pod-filling were abandoned. Inoculation treatments produced similar results for both desi and kabuli chickpeas at all locations. Therefore, genotype data were averaged over locations for each year. With the exception of for the Outlook plots in 1997 (Appendices 2 and 3), limited (though sparse) nodulation occurred on non-inoculated plots (appendices 4-19), despite the care taken to avoid contamination.

Number of nodules per plant in 1997: Inoculation treatments and depth of inoculant placement significantly influenced numbers of nodules and nodulation patterns in both desi and kabuli chickpeas at all locations. For the 1997 growing season, averaged over locations, the peat-based inoculants produced more nodules per plant than for the liquid inoculants at both sampling dates in both desi chickpea (Tables 3.3-3.6) and kabuli chickpea (Tables 3.7-3.10). Furthermore, the average number of nodules for the liquid + peat-based inoculants was higher than the average for the six granular inoculants at both sampling dates. In the desi experiments, these differences in nodule numbers were significant at the 5% level at the flowering stage (Table 3.3), but the differences increased as the plants approached the early pod-filling stage (Table 3.4), and were significant at the 1% level.

In 1997, the total nodule numbers for the granular inoculants applied in the seed furrow were significantly higher than when the granular inoculants were placed below the seed at both sampling dates in desi chickpea (Tables 3.3-3.6), but the differences were significant only at the early pod-filling stage in the kabuli chickpea (Tables 3.7–3.10). Again, the differences in the desi chickpea increased from the flowering stage (P = 0.05) to the early pod-filling stage (P = 0.01). However, no significant differences in total nodule numbers were observed between the granular inoculant placed in the seed furrow and the peat-based inoculant. Rhizobial strain or strains in the same formulation did not differ in number of nodules and the strain interactions were not significant in either the desi or the kabuli chickpeas. Furthermore, the depth of placement of the granular inoculant (2.5 cm and 8.0 cm below the seed) had no effect on nodule numbers.

In 1997, the location x inoculation interaction for the total number of nodules per plant was significant only at the early pod-filling stage for the desi chickpea (Table 3.6) and only at the late pod-filling stage for the kabuli chickpea (Table 3.10), due primarily to the higher number of nodules for the liquid inoculant at Watrous for the desi chickpea (Appendix 4) and the low number of nodules for the liquid inoculant at Watrous for the kabuli chickpea (Appendix 5), relative to the peat-based inoculant. The significant differences in total number of nodules for the desi chickpea and the kabuli chickpea in 1997 reported above are due primarily to differences in number of

nodules in the crown area (Tables 3.3-3.10). Very few of the differences in number of nodules on the lateral roots were significant.

Location had a significant effect on number of nodules in the desi experiments (Tables 3.5 and 3.6), but the effect was not significant in the kabuli experiments (Tables 3.8 and 3.10). Total nodule numbers at both the flowering and early pod-filling stages for desi chickpea at Outlook (Appendices 2 and 3), Kenaston (Appendices 6 and 7) and Watrous locations (Appendices 4 and 8) were generally two to three and half times greater than those recorded at Elbow (Appendices 9 and 10). On the other hand, total nodule numbers for the kabuli chickpea were similar at Watrous (Appendices 5 and 11) and Kenaston (Appendices 12 and 13).

Nodule dry weight in 1997: For the 1997 experiments, nodule dry weight was often not consistent with the number of nodules produced in either the desi or the kabuli experiments at all locations (Appendices 2-13). Differences in number of nodules plant^{-1} often were not detected as differences in nodule dry weight plant^{-1}. For example, in the desi chickpea experiments, granular inoculants placed below the seed produced a lower number of nodules, but the total dry weights were not significantly different from those for the peat inoculants (Tables 3.3-3.6). The orthogonal contrast of liquid + peat vs. granular inoculant treatment indicated no significant differences in the nodule dry weight. Total nodule dry weights for the liquid inoculants were lower than that for the peat inoculants at both the flowering and the early pod-filling stages in desi chickpea (Tables 3.3 and 3.4). At the flowering stage, total nodule dry weight for the granular inoculant placed 2.5 cm below the seed was significantly (P = 0.03) higher as compared to that for placement 8.0 cm below the seed. Total nodule dry weight for granular B inoculants at the early pod-filling stage in the desi was significantly (P = 0.01) higher than for granular A inoculants.

Table 3.3. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage, averaged over the Elbow, Kenaston, Outlook and Watrous locations, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoc	0.15	1.09	1.24	2.3	2.9	5.2	1.23
Liq A	1.38	2.43	3.80	18.4	10.1	28.5	1.29
Liq C	1.18	3.28	4.45	22.9	11.6	34.5	1.19
Peat A	1.85	3.09	4.94	33.8	20.4	54.2	1.22
Peat B	3.03	3.33	6.35	43.9	15.9	59.8	1.29
Gran A with seed	1.35	2.21	3.56	21.4	27.0	48.4	1.43
Gran A 2.5 cm bs	0.40	3.33	3.73	10.8	42.3	53.0	1.41
Gran A 8.0 cm bs	0.34	2.11	2.45	7.0	36.5	43.5	1.33
Gran B with seed	1.56	4.51	6.08	18.4	29.0	47.4	1.24
Gran B 2.5 cm bs	0.58	3.18	3.75	18.1	47.0	65.1	1.36
Gran B 8.0 cm bs	0.36	2.55	2.91	6.6	29.3	35.9	1.40
$LSD_{(0.05)}$	0.99	1.69	1.98	19.0	15.7	23.9	ns
Contrasts[‡]							
Non-inoc vs. inoc	1.05**	1.91**	2.96**	17.8 *	24.0**	41.8**	0.09
Liq vs. peat	1.16**	0.36	1.52*	18.2**	7.3	25.5**	0.02
Liq A vs. liq C	0.20	0.85	0.65	4.5	1.5	6.0	0.10
Liq vs. gran	0.58	0.13	0.38	6.9	24.3**	17.4*	0.12
Peat A vs. peat B	1.18*	0.24	1.41	10.1	4.5	5.6	0.07
Peat vs. gran	1.68**	0.24	1.90**	25.1**	17.0**	8.1	0.11
Liq+peat vs. gran	1.10**	0.05	1.14*	16.0**	20.7**	4.6	0.11*
Gran ws vs. gran bs	1.04**	0.57	1.61*	9.3	10.8*	1.5	0.04
Gran 2.5 vs. gran 8.0	0.14	0.93	1.06	7.7	11.8*	19.4*	0.02
Gran A vs. gran B	0.14	0.86	1.00	1.3	0.2	1.2	0.06
Gran str x ws vs. bs	0.02	0.15	0.13	6.1	7.2	13.2	0.08
Gran str x 2.5 vs. 8.0	0.08	0.30	0.22	3.9	6.0	9.9	0.06

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain

[‡] Differences between specified treatments.

Table 3.4. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage, averaged over the Elbow, Kenaston, Outlook and Watrous locations, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoc	0.23	0.55	0.78	6.6	17.6	24.2	3.71
Liq A	2.28	1.51	3.79	47.8	26.6	74.4	3.87
Liq C	3.05	1.36	4.41	65.6	21.9	87.5	4.02
Peat A	3.76	1.76	5.53	88.0	42.4	130.4	4.14
Peat B	4.31	1.81	6.13	100.1	30.0	130.1	4.00
Gran A with seed	2.00	2.24	4.24	55.9	51.4	107.3	4.36
Gran A 2.5 cm bs	0.59	2.25	2.84	22.4	99.5	121.9	4.88
Gran A 8.0 cm bs	0.38	2.08	2.45	6.1	78.4	84.5	4.54
Gran B with seed	2.48	3.36	5.84	82.5	55.1	137.6	4.72
Gran B 2.5 cm bs	0.78	2.42	3.20	30.0	120.8	150.8	4.68
Gran B 8.0 cm bs	0.61	2.60	3.21	29.6	104.9	134.5	5.28
$LSD_{(0.05)}$	1.51	1.08	1.87	40.7	39.8	46.2	0.71
Contrasts[‡]							
Non-inoc vs. inoc	1.79**	1.59**	3.38**	46.2**	45.5**	91.7**	0.74**
Liq vs. peat	1.37**	0.35	1.73**	37.4**	12.0	49.3**	0.13
Liq A vs. liq C	0.77	0.15	0.62	17.8	4.7	13.1	0.15
Liq vs. gran	1.53**	1.06**	0.47	19.0	60.8**	41.8**	0.80 **
Peat A vs. peat B	0.55	0.05	0.60	12.1	12.4	0.3	0.14
Peat vs. gran	4.04**	0.71*	2.20**	56.3**	48.9**	7.5	0.67**
Liq+peat vs. gran	2.21**	0.88**	1.33**	37.6**	54.8**	17.2	0.74**
Gran ws vs. gran bs	1.65**	0.46	2.11**	47.2**	47.7**	0.5	0.31
Gran 2.5 vs. gran 8.0	0.19	0	0.19	8.3	18.5	26.9	0.13
Gran A vs. gran B	0.30	0.60*	0.91	19.2	17.2	36.4**	0.30
Gran str x ws vs. bs	0.03	0.38	0.41	3.3	11.1	7.8	0.21
Gran str x 2.5 vs. 8.0	0.03	0.18	0.20	8.0	2.6	10.6	0.47

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

[‡] Differences between specified treatments.

Table 3.5. Mean squares from the analysis of variance for number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage, at the Elbow, Kenaston, Outlook and Watrous locations, 1997.

		Mean squares						
		Nodule number plant^{-1}			Nodule dry wt.			Shoot dry wt.
Source of variation†	d.f.	Crown	Lateral	Total	Crown	Lateral	Total	
Locations (L)	3	6.48*	85.99**	116.75**	0.005**	0.008**	0.021**	4.266**
Reps in locations	12	0.75	1.37	2.74	0.000	0.000	0.001	0.262**
Inoculation (I)	10	11.87**	12.61*	35.91**	0.002**	0.003**	0.004**	0.110
Non-inoc vs. inoc	1	16.07**	53.20**	127.76**	0.005*	0.008**	0.026**	0.095
Liq vs. peat	1	21.62**	2.03	36.91*	0.005**	0.001	0.010**	0.003
Liq A vs. liq C	1	0.32	5.78	3.38	0.000	0.000	0.000	0.070
Liq vs. gran	1	6.25	0.41	3.45	0.001	0.014**	0.007*	0.346
Peat A vs. peat B	1	11.05*	0.45	15.96	0.001	0.000	0.000	0.043
Peat vs. gran	1	67.17**	1.21	86.45**	0.015**	0.007**	0.002	0.267
Liq+peat vs. gran	1	45.76**	0.08	49.78*	0.010**	0.016**	0.001	0.489*
Gran ws vs. gran bs	1	22.96**	6.98	55.26*	0.002	0.003*	0.000	0.032
Gran 2.5 vs. gran 8	1	0.30	13.51	17.85	0.001	0.002*	0.006*	0.005
Gran A vs. gran B	1	0.45	17.85	24.00	0.000	0.000	0.000	0.077
Gran str x ws vs. bs	1	0.03	10.18	11.31	0.001	0.001	0.002	0.235
Gran str x 2.5 vs. 8	1	0.09	1.38	0.77	0.000	0.001	0.002	0.061
L x I	30	1.86**	5.49	7.53	0.001**	0.000	0.001*	0.115**
Error	120	0.83	4.91	6.02	0.000	0.000	0.001	0.063
Total	175							

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 3.6. Mean squares from the analysis of variance for number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage, at the Elbow, Kenaston, Outlook and Watrous locations, 1997.

		Mean squares						
		Nodule number plant^{-1}			Nodule dry wt.			Shoot dry wt.
Source of variation[†]	d.f.	Crown	Lateral	Total	Crown	Lateral	Total	
Locations (L)	3	42.16*	52.30**	184.55**	0.027	0.023**	0.100**	58.469**
Reps in locations	12	6.60**	1.09	10.11**	0.009**	0.003*	0.015**	4.564**
Inoculation (I)	10	33.36**	8.58**	41.07**	0.017**	0.021**	0.022**	3.742**
Non-inoc vs. inoc	1	47.00**	36.77**	166.91**	0.031**	0.030**	0.122**	7.947**
Liq vs. peat	1	30.25**	1.96	47.61**	0.022**	0.002	0.039**	0.245
Liq A vs. liq C	1	4.81	0.18	3.13	0.003	0.000	0.001	0.189
Liq vs. gran	1	55.82**	26.67**	5.32	0.009	0.089**	0.042**	15.293**
Peat A vs. peat B	1	2.42	0.02	2.88	0.001	0.001	0.000	0.179
Peat vs. gran	1	201.84**	11.06*	115.72**	0.076**	0.057**	0.001	10.919**
Liq+peat vs. gran	1	187.97**	29.68**	68.27**	0.054**	0.115**	0.011	20.822**
Gran ws vs. gran bs	1	58.08**	4.56	95.20**	0.047**	0.048**	0.000	1.971
Gran 2.5 vs. gran 8	1	0.56	0.00	0.56	0.001	0.005	0.012	0.264
Gran A vs. gran B	1	2.16	8.88*	19.80	0.009	0.007	0.032**	2.151
Gran str x ws vs. bs	1	0.44	6.75	10.64	0.002	0.001	0.000	1.278
Gran str x 2.5 vs. 8	1	0.01	0.49	0.64	0.001	0.011	0.002	3.608
L x I	30	4.39**	2.22**	6.74**	0.003**	0.003**	0.004	0.963
Error	120	1.19	1.21	2.68	0.001	0.001	0.003	0.934
Total	175							

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 3.7. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stages, averaged over the Kenaston and Watrous locations, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g pl^{-1})[§]
Non-inoc	0.45	0.43	0.88	9.5	13.0	22.5	6.18
Liq A	2.60	2.00	4.60	64.3	58.0	122.2	6.24
Liq C	3.90	1.98	5.88	80.3	53.5	133.8	6.14
Peat A	5.73	3.03	8.75	178.8	67.0	245.8	6.55
Peat B	7.33	4.20	11.53	221.3	88.0	309.3	8.14
Gran A with seed	2.93	5.53	8.45	82.0	116.3	198.3	7.32
Gran A 2.5 cm bs	0.50	5.48	5.98	20.3	174.3	194.5	8.94
Gran A 8.0 cm bs	0.40	5.33	5.73	4.3	122.8	127.0	8.25
Gran B with seed	2.25	5.55	7.80	38.0	123.0	161.0	7.69
Gran B 2.5 cm bs	0.75	4.35	5.10	22.5	162.5	185.0	8.83
Gran B 8.0 cm bs	0.43	4.70	5.13	28.3	184.8	213.0	8.64
$LSD_{(0.05)}$	1.65	3.19	3.60	71.5	47.1	75.8	1.49
Contrasts[‡]							
Non-inoc vs. inoc	2.23**	3.79**	6.02**	64.5*	102.0**	166.5**	1.49*
Liq vs. peat	3.28**	1.63	4.90**	127.8**	21.8	149.6**	1.16*
Liq A vs. liq C	1.30	0.02	1.28	16.0	4.5	11.6	0.10
Liq vs. gran	2.04**	3.17**	1.13	39.7	91.5**	51.8 *	2.09**
Peat A vs. peat B	1.60	1.17	2.78	42.5	21.0	63.5	1.59*
Peat vs. gran	5.32**	1.54	3.78**	167.5**	69.8 **	97.8**	0.93*
Liq+peat vs. gran	3.68**	2.35**	1.33	103.6**	80.7**	23.0	1.51**
Gran ws vs. gran bs	2.07**	0.58	2.64*	41.2	41.5**	0.2	1.16*
Gran 2.5 vs. gran 8.0	0.21	0.10	0.11	5.1	14.6	19.8	0.44
Gran A vs. gran B	0.13	0.58	0.71	5.9	19.0	13.1	0.22
Gran str x ws vs. bs	0.37	0.07	0.29	18.1	7.5	25.6	0.17
Gran str x 2.5 vs. 8.0	0.11	0.25	0.14	10.9	36.9*	47.8	0.25

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

[‡] Differences between specified treatments.

[§] g pl^{-1} = g plant^{-1}

Table 3.8. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stages, averaged over the Kenaston and Watrous locations, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g pl^{-1})§
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoc	0.48	0.50	0.98	18.0	33.0	51.0	8.77
Liq A	2.25	2.58	4.83	67.8	77.0	144.8	10.30
Liq C	3.58	2.23	5.80	104.8	123.5	228.3	9.16
Peat A	6.00	4.35	10.35	175.3	105.5	280.8	12.31
Peat B	5.30	5.45	10.75	184.8	136.5	321.3	12.54
Gran A with seed	2.95	5.38	8.33	76.8	154.0	230.8	12.49
Gran A 2.5 cm bs	0.78	7.03	7.80	34.0	179.8	213.8	15.57
Gran A 8.0 cm bs	0.15	4.98	5.13	2.3	178.3	180.5	14.20
Gran B with seed	1.93	5.75	7.68	42.5	145.0	187.5	12.12
Gran B 2.5 cm bs	0.48	4.75	5.23	19.8	204.3	224.0	13.46
Gran B 8.0 cm bs	0.13	4.03	4.15	9.5	169.8	179.3	15.34
$LSD_{(0.05)}$	1.59	3.77	4.54	57.4	91.4	118.0	4.18
Contrasts‡							
Non-inoc vs. inoc	1.87**	4.15**	6.03**	53.7*	114.4**	168.1**	3.98*
Liq vs. peat	2.34**	2.50	5.24**	93.8**	20.8	114.5*	2.70*
Liq A vs. liq C	1.33	0.35	0.97	37.0	46.5	83.5	1.14
Liq vs. gran	1.85**	2.92**	1.07	55.5 **	71.6**	16.1	4.13**
Peat A vs. peat B	0.70	1.10	0.40	9.5	31.0	40.5	0.23
Peat vs. gran	4.58**	0.42	4.16**	149.2**	50.9	98.4**	1.44
Liq+peat vs. gran	3.21**	1.67	1.55	102.4**	61.2**	41.2	2.79**
Gran ws vs. gran bs	2.06**	0.37	2.43	43.3*	33.6	9.8	2.34
Gran 2.5 vs. gran 8.0	0.49	1.39	1.88	21.0	18.0	39.0	0.26
Gran A vs. gran B	0.45	0.95	1.40	13.8	2.3	11.4	0.45
Gran str x ws vs. bs	0.67	0.80	1.47	25.4	15.0	40.4	0.05
Gran str x 2.5 vs. 8.0	0.14	0.67	0.80	10.7	16.5	5.7	1.63

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

‡ Differences between specified treatments.

§ g pl^{-1} = g plant^{-1}

Table 3.9. Mean squares from the analysis of variance for number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stages, at the Kenaston and Watrous locations, 1997.

		Mean squares						
		Nodule number plant^{-1}			Nodule dry wt.			Shoot dry wt.
Source of variation†	d.f.	Crown	Lateral	Total	Crown	Lateral	Total	
Locations (L)	1	9.69	8.16	35.64	0.002	0.011	0.023	64.15*
Reps in locations	6	6.94*	8.69*	18.51*	0.007	0.002	0.011	6.30
Inoculation (I)	10	44.70**	24.76*	60.73**	0.040**	0.024**	0.045**	9.77**
Non-inoc vs. inoc	1	36.17**	104.33**	263.35**	0.030*	0.076**	0.202**	16.14*
Liq vs. peat	1	85.81**	21.13	192.08**	0.131**	0.004	0.179**	10.55*
Liq A vs. liq C	1	6.76	0.01	6.50	0.001	0.0001	0.001	0.04
Liq vs. gran	1	50.02**	120.33**	15.19	0.019	0.100**	0.032*	52.12**
Peat A vs. peat B	1	10.24	5.52	30.80	0.007	0.002	0.016	10.09*
Peat vs. gran	1	339.20**	28.52	171.01**	0.337**	0.058**	0.115**	10.51*
Liq+peat vs. gran	1	259.90**	106.41**	33.71	0.206**	0.125**	0.010	43.77**
Gran ws vs. gran bs	1	45.65**	3.53	74.55*	0.018	0.018**	0.000	14.29*
Gran 2.5 vs. gran 8	1	0.36	0.08	0.10	0.0002	0.002	0.003	1.56
Gran A vs. gran B	1	0.21	3.97	6.02	0.0004	0.004	0.002	0.56
Gran str x ws vs. bs	1	2.10	0.04	1.55	0.006	0.000	0.009	0.01
Gran str x 2.5 vs. 8	1	0.10	0.50	0.15	0.001	0.011*	0.018	0.51
L x I	10	21.93	8.21*	10.45	0.004	0.002	0.005	1.79
Error	60	2.63	3.80	6.92	0.004	0.003	0.007	3.05
Total	87							

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 3.10. Mean squares from the analysis of variance for number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stages, at the Kenaston and Watrous locations, 1997.

		Mean squares						
		Nodule number plant^{-1}			Nodule dry wt.			Shoot dry wt.
Source of variation[†]	d.f.	Crown	Lateral	Total	Crown	Lateral	Total	
Locations (L)	1	3.52	22.00	7.92	0.002	0.146**	0.181*	71.47
Reps in locations	6	2.36*	6.33**	12.16**	0.004	0.006	0.015*	11.82*
Inoculation (I)	10	34.45**	27.56*	65.70*	0.033**	0.020*	0.040*	41.00*
Non-inoc vs. inoc	1	25.64**	125.26**	264.22**	0.021*	0.095**	0.205**	114.85*
Liq vs. peat	1	59.95**	50.00	219.45**	0.070**	0.003	0.105*	58.07
Liq A vs. liq C	1	7.02	0.49	3.80	0.005	0.004	0.028	5.16
Liq vs. gran	1	40.89**	102.08**	13.76	0.037**	0.061**	0.003	205.22**
Peat A vs. peat B	1	1.96	4.84	0.64	0.001	0.012	0.007	0.21
Peat vs. gran	1	252.08**	2.08	208.33**	0.267**	0.031	0.116**	24.92
Liq+peat vs. gran	1	198.40**	53.33	46.00	0.201**	0.072**	0.032	149.27**
Gran ws vs. gran bs	1	14.08**	1.45	62.73	0.020*	0.012	0.001	58.30
Gran 2.5 vs. gran 8	1	1.56	15.40	28.13	0.003	0.003	0.012	0.53
Gran A vs. gran B	1	2.43	10.83	23.52	0.002	0.001	0.001	2.37
Gran str x ws vs. bs	1	6.10	2.73	17.00	0.008	0.002	0.018	0.04
Gran str x 2.5 vs. 8	1	0.15	3.51	5.12	0.001	0.002	0.000	21.22
L x I	10	2.05*	11.45**	16.64**	0.003	0.007	0.011	14.03**
Error	60	0.98	1.94	3.71	0.002	0.004	0.006	5.24
Total	87							

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

For the kabuli experiments in 1997, analysis by contrast showed that the peat inoculants produced a higher total nodule dry weight than the liquid or granular inoculants at both the early pod-filling stage (Table 3.7) and the late pod-filling stage (Table 3.8). However, total nodule dry weight for the granular inoculant was higher than for the liquid inoculant at the early pod-filling stage.

The interaction between inoculation treatment and location was not significant for total nodule dry weight for either chickpea type at either sampling date (Tables 3.5, 3.6, 3.9 and 3.10), except for desi at the flowering stage, presumably due to the higher nodule dry weight for the liquid inoculation at Watrous (Appendix 8). Except for the early pod-filling stage in the kabuli experiments, location had a significant effect on nodule dry weight due to the low nodule dry weight at Elbow (Appendices 9 and 10) and Outlook (Appendices 2 and 3) in the desi experiments, and the higher nodule dry weight at Kenaston (Appendix 13) as compared to Watrous (Appendix 5) in the kabuli experiments at the late pod-filling stage. The greatest nodule dry weight (263 mg plant^{-1}) in the desi experiments occurred with granular B inoculant placed 2.5 cm below the seed at Watrous at the early pod-filling stage (Appendix 4). For the kabuli experiments, the greatest nodule dry weight was 389.5 mg plant^{-1} for peat B inoculant at Kenaston during the late pod-filling stage (Appendix 13).

Number of nodules in 1998: Unlike the 1997 field season, the 1998 results at Outlook and Watrous indicated that the granular inoculants produced more nodules than the average of the peat and the liquid inoculants at the early pod-filling stage in both chickpea types (Tables 3.11-3.14). However, seed treatment with peat-based inoculants resulted in higher nodule numbers as compared to the liquid inoculants. Liquid A performed poorly and was not significantly different from the non-inoculated control in both the desi and kabuli chickpeas at the early pod-filling stage. Liquid B produced more nodules than liquid A at the early pod-filling stage in the kabuli experiment (Table 3.12), but not for the desi experiment (Table 3.11).

The interaction between location and inoculation was not significant for total nodule numbers at the early pod-filling stage in either the desi or kabuli experiment (Tables 3.13 and 3.14). Similarly, location had no effect on total number of nodules.

Sampling at the late pod-filling stage was performed at Watrous only due to dry soil conditions at Outlook, which made it difficult to excavate and recover roots and attached nodules. At the late pod-filling stage, the total nodule numbers at Watrous were similar to those observed at the early pod-filling stage in both chickpea cultivars, and followed a trend similar to that in 1997 (Appendices 14 and 15).

Nodule dry weight in 1998: The total dry weight of the nodules at the early pod-filling stage was greater in the peat and granular than the liquid inoculation treatments in both chickpea types (Tables 3.11-3.14). For the desi experiments, no significant difference in nodule dry weight was observed for the peat vs. granular inoculant, but the difference was significant in the kabuli experiments. Liquid B inoculant produced more total nodule dry weight than liquid A inoculant at the early pod-filling stage in the desi experiments (Table 3.11), but not in the kabuli experiments (Table 3.12). For the kabuli chickpea, granular inoculant B placed with the seed resulted in higher total nodule dry weight as compared to treatments in which the granular inoculant B was placed below the seed (Table 3.12). For both chickpea types, total nodule dry weight at the early pod-filling stage for the liquid A inoculant was not significantly different from the non-inoculated control.

In 1998, no significant interaction was observed between location and inoculation for total nodule dry weight at the early pod-filling stage in the desi experiments (Table 3.13), but the interaction was significant ($P = 0.02$) in the kabuli experiments (Table 3.14), presumably due to the extremely low total nodule dry weight for the liquid inoculants at Outlook. The effect of location on nodule dry weight was not significant for either the desi or the kabuli chickpeas. Desi chickpea plants grown from seeds inoculated with peat A inoculant produced the greatest total nodule dry weight (307.5 mg plant^{-1}) at the early pod-filling stage at Outlook (Appendix 16), whereas granular A placed 8 cm below the seed resulted in the highest nodule dry weight (275.0 mg plant^{-1}) at the early pod-filling stage at Watrous (Appendix 17). For the kabuli chickpea, the highest nodule dry weight at Watrous occurred in the peat B treatment (317.5 mg plant^{-1}) (Appendix 18), whereas at Outlook the highest nodule weight of 206.0 mg plant^{-1} was achieved for the peat A treatment

(Appendix 19). At the late pod-filling stage, total nodule dry weight for the desi chickpea at Watrous (Appendix 14), unlike the kabuli chickpea (Appendices 15 and 18), was generally lower as compared to that observed at the early pod-filling stage (Appendix 17).

Table 3.11. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage, averaged over the Outlook and Watrous locations, 1998.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g pl^{-1})[§]
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoc	0	0.13	0.13	0	3.0	3.0	4.70
Liq A	0.18	0.35	0.53	9.5	16.0	25.5	4.16
Liq B	2.00	1.40	3.40	76.3	48.0	124.3	4.73
Peat A	3.27	2.38	5.65	193.3	70.8	264.0	5.13
Peat B	3.88	2.30	6.18	150.5	41.0	191.5	5.39
Gran A with seed	2.43	5.45	7.88	80.3	163.0	243.3	6.26
Gran A 2.5 cm bs	1.13	6.23	7.35	21.8	182.3	204.0	5.94
Gran A 8.0 cm bs	0.40	6.70	7.10	7.8	196.3	204.0	7.03
Gran B with seed	2.13	4.70	6.83	87.3	134.5	221.8	5.59
Gran B 2.5 cm bs	1.08	6.10	7.18	25.5	131.5	157.0	5.91
Gran B 8.0 cm bs	0.60	4.95	5.55	19.0	134.5	153.5	6.33
$LSD_{(0.05)}$	1.72	1.92	3.03	45.9	76.2	94.3	0.97
Contrasts[‡]							
Non-inoc vs. inoc	1.71*	3.93**	5.64**	67.1*	108.8**	175.9**	0.95**
Liq vs. peat	2.49**	1.47**	3.95**	129.0**	23.9	152.9**	0.82*
Liq A vs. liq B	1.82*	1.05	2.87	66.8**	32.0	98.8*	0.57
Liq vs. gran	0.21	4.81**	5.02**	2.6	125.0**	122.4**	1.74**
Peat A vs. peat B	0.61	0.08	0.53	42.8	29.8	72.5	0.26
Peat vs. gran	2.28**	3.35**	1.07	131.6**	101.1**	30.5	0.92**
Liq+peat vs. gran	1.04**	4.08**	3.04**	67.1**	113.1**	45.9*	1.32**
Gran ws vs. gran bs	1.48**	0.92	0.56	65.3**	12.4	52.9	0.38
Gran 2.5 vs. gran 8.0	0.61	0.34	0.94	10.3	8.5	1.8	0.76*
Gran A vs. gran B	0.05	0.88	0.92	7.3	47.0*	39.7	0.47
Gran str x ws vs. bs	0.50	0.48	0.98	4.5	3.8	8.3	0.28
Gran str x 2.5 vs. 8.0	0.13	0.81	0.69	3.8	5.5	1.8	0.34

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

[‡] Differences between specified treatments.

[§] g pl^{-1} = g plant^{-1}

Table 3.12. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage, averaged over the Outlook and Watrous locations, 1998.

Inoculant[†]	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g pl^{-1})[§]
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoc	0.05	0.50	0.55	1.5	21.5	23.0	6.53
Liq A	0.55	1.13	1.68	26.5	50.8	77.3	7.78
Liq B	2.45	2.73	5.18	69.3	73.0	142.3	6.39
Peat A	3.25	2.68	5.93	144.0	90.3	234.3	8.39
Peat B	4.88	3.25	8.13	165.0	79.3	244.3	9.30
Gran A with seed	2.50	5.05	7.55	59.0	115.0	174.0	7.86
Gran A 2.5 cm bs	0.88	8.38	9.25	16.0	164.5	180.5	8.86
Gran A 8.0 cm bs	0.58	7.90	8.48	8.8	173.0	181.8	8.57
Gran B with seed	3.00	5.10	8.10	92.3	150.8	243.0	8.97
Gran B 2.5 cm bs	1.40	6.00	7.40	35.3	133.5	168.8	9.02
Gran B 8.0 cm bs	0.20	6.50	6.70	10.3	137.3	147.5	8.31
$LSD_{(0.05)}$	1.57	2.12	2.58	56.4	76.5	86.7	1.90
Contrasts[‡]							
Non-inoc vs. inoc	1.92**	4.37**	6.29**	61.2**	95.3**	156.4**	1.82*
Liq vs. peat	2.57**	1.04	3.60**	106.6**	22.9	129.5**	1.76*
Liq A vs. liq B	1.90*	1.60	3.50**	42.8	22.2	65.0	1.39
Liq vs. gran	0.07	4.56**	4.48**	11.0	83.8**	72.8**	1.51**
Peat A vs. peat B	1.63*	0.57	2.20	21.0	22.0	10.0	0.91
Peat vs. gran	2.64**	3.52**	0.88	117.6**	60.8**	56.7*	0.25
Liq+peat vs. gran	1.36**	4.04**	2.68**	64.3**	72.3**	8.1	0.63
Gran ws vs. gran bs	1.99**	2.12**	0.13	58.1**	19.2	38.9	0.28
Gran 2.5 vs. gran 8.0	0.75	0.01	0.74	16.1	6.2	10.0	0.50
Gran A vs. gran B	0.21	1.24*	1.03	18.0	10.3	7.7	0.34
Gran str x ws vs. bs	0.33	0.02	0.31	0.4	16.0	16.3	0.37
Gran str x 2.5 vs. 8.0	0.45	0.49	0.04	8.9	2.4	2.4	0.21

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

[‡] Differences between specified treatments.

[§] g pl^{-1} = g plant^{-1}

Table 3.13. Mean squares from the analysis of variance for number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage, at the Outlook and Watrous locations, 1998.

Source of variation[†]	d.f.	Mean squares						
		Nodule number plant^{-1}			Nodule dry wt.			Shoot dry wt.
		Crown	Lateral	Total	Crown	Lateral	Total	
Locations (L)	1	1.92	0.10	1.14	0.005	0.003	0.016	28.25
Reps in locations	6	1.93*	8.32*	17.84**	0.002	0.006	0.009	5.29*
Inoculation (I)	10	13.28**	47.82**	59.22**	0.032**	0.037**	0.056**	5.70**
Non-inoc vs. inoc	1	21.20*	112.33**	231.14**	0.033**	0.086**	0.225**	6.58**
Liq vs. peat	1	49.50**	17.11*	124.82**	0.133**	0.005	0.187**	5.29*
Liq A vs. liq B	1	13.32*	4.41	33.06	0.018**	0.004	0.039*	1.31
Liq vs. gran	1	0.50	277.92**	302.00**	0.000	0.188**	0.180**	36.00**
Peat A vs. peat B	1	1.44	0.02	1.10	0.007	0.004	0.021	0.26
Peat vs. gran	1	62.56**	134.67**	13.65	0.208**	0.123**	0.011	10.13**
Liq+peat vs. gran	1	20.75**	319.81**	177.63**	0.087**	0.245**	0.401*	33.72**
Gran ws vs. gran bs	1	23.21**	9.00	3.30	0.045**	0.002	0.030	1.50
Gran 2.5 vs. gran 8	1	2.88	0.91	7.03	0.001	0.001	0.000	4.55*
Gran A vs. gran B	1	0.03	9.19	10.27	0.001	0.027*	0.019	2.62
Gran str x ws vs. bs	1	2.16	3.15	10.53	0.000	0.001	0.001	0.02
Gran str x 2.5 vs. 8	1	0.13	5.28	3.78	0.000	0.000	0.000	0.89
L x I	10	2.39**	2.98	7.39	0.002	0.005	0.007	0.76
Error	60	0.76	3.14	4.07	0.003	0.003	0.004	2.10
Total	87							

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 3.14. Mean squares from the analysis of variance for number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage, at the Outlook and Watrous locations, 1998.

Source of variation[†]	d.f.	Mean squares						
		Nodule number plant[-1]			Nodule dry wt.			Shoot dry wt.
		Crown	Lateral	Total	Crown	Lateral	Total	
Locations (L)	1	35.89	93.69	245.56	0.034	0.077	0.214	270.16**
Reps in locations	6	0.55	1.58	2.61	0.001	0.001	0.004	9.18**
Inoculation (I)	10	18.89	55.13	63.03	0.025	0.019	0.037	7.55
Non-inoc vs. inoc	1	26.74**	138.89**	287.51**	0.027**	0.066**	0.178**	23.89*
Liq vs. peat	1	52.53**	8.61	103.68**	0.091**	0.004	0.134**	24.77*
Liq A vs. liq B	1	14.44*	10.24	49.00**	0.007	0.002	0.017	7.80
Liq vs. gran	1	0.07	249.80**	241.65**	0.001	0.084**	0.064**	27.49**
Peat A vs. peat B	1	10.56*	1.32	9.36	0.002	0.001	0.001	3.30
Peat vs. gran	1	83.48**	149.11**	9.45	0.166**	0.045**	0.039*	0.73
Liq+peat vs. gran	1	35.32**	313.96**	138.68**	0.079**	0.101**	0.001	7.71
Gran ws vs. gran bs	1	42.14**	47.88**	0.18	0.036**	0.004	0.016	0.78
Gran 2.5 vs. gran 8	1	4.50	0.01	4.35	0.002	0.001	0.001	2.00
Gran A vs. gran B	1	0.56	18.50*	12.61	0.004	0.001	0.001	1.37
Gran str x ws vs. bs	1	0.17	0.01	0.09	0.001	0.004	0.009	0.99
Gran str x 2.5 vs. 8	1	1.62	1.90	0.01	0.001	0.000	0.001	0.36
L x I	10	1.99	3.63	5.36	0.003	0.005	0.006*	2.90
Error	60	0.49	4.29	4.84	0.001	0.002	0.051	2.58
Total	87							

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Nodule distribution in 1997: Inoculum placement significantly affected the distribution of nodules on the root system and the distribution was consistent across locations in both chickpea types. The peat and liquid inoculants produced majority of the nodules at the crown region, whereas the soil-applied (granular) inoculants produced mainly lateral root nodules, especially when the granular inoculum was placed below the seed (e.g., Tables 3.4 and 3.7). In the desi experiments averaged over locations for the 1997 field season, granular inoculant placed at 2.5 and 8.0 cm below the seed formed 72-97% of the nodules on the lateral roots (on nodule dry weight basis) compared to only 25-36% for the peat and liquid inoculants at the flowering and early pod-filling stages (Tables 3.3 and 3.4, respectively). Similarly, 87-97% of the nodules formed by granular inoculant placed below the seed in kabuli were located on the lateral roots compared to 27-54% for the peat and liquid inoculants at the early and late pod-filling stages (Tables 3.7 and 3.8, respectively).

Nodule distribution in 1998: The position of the nodules in 1998 experiments was similar to that in 1997. For example, based on dry weight, granular inoculants placed below the seed produced 79-96% of their nodules on the lateral roots in both chickpea types at the early pod-filling stage as compared to 21-39% in the peat inoculants (Tables 3.11 and 3.12). There were no marked differences among inoculant strains in either chickpea type in both years indicating that the pattern of nodule formation was due primarily to the depth of inoculant placement.

3.3.1.2 Dry matter yield

1997: At all sampling dates, averaged over locations, shoot dry matter was significantly affected by inoculation methods except for the flowering stage in desi chickpea, even though a similar trend was observed (Tables 3.3-3.10). For the 1997 experiments, inoculation generally increased shoot dry matter per plant compared to the control, but dry matter yield increases were higher with the granular inoculants placed below the seed than when placed in the seed row or for peat and liquid inoculants. Orthogonal contrasts confirmed that shoot dry weight in both desi and kabuli chickpeas were significantly higher for soil inoculation as compared to seed

inoculation (Tables 3.3-3.10). Moreover, whereas the differences were detected at the 5% level in the desi chickpea at the flowering stage (Table 3.3), the significance increased to the 1% level at the early pod-filling stages (Table 3.4). Shoot dry weight for the kabuli plants grown from seeds treated with peat-based inoculants was significantly higher than that for the liquid formulated treatments at the early pod-filling stage (Table 3.7) and at the late pod-filling stage (Table 3.8). Peat B inoculation resulted in higher shoot dry matter production than peat A inoculation in the kabuli chickpea at both the early pod-filling stage (Table 3.7) and the late pod-filling stage (Table 3.8).

The interaction between location and inoculation for shoot dry weight was not significant at the early pod-filling stage (Tables 3.6 and 3.9) in either chickpea type. However, a significant interaction was observed in the desi at flowering (Table 3.5), presumably due to lack of significant differences among inoculation treatments at Elbow (Appendix 9) relative to significant differences at the other three sites (Appendices 2, 6 and 8). A significant interaction was also observed in the kabuli at late pod-filling (Table 3.10), presumably due to the low shoot dry matter for the granular B inoculant placed with the seed in 1997 at Watrous (Appendix 5) relative to the high dry matter yield at Kenaston (Appendix 13). Location had a significant effect on shoot dry matter at both sampling dates in the desi chickpea (Tables 3.5 and 3.6) but was significant only at the early pod-filling stage in the kabuli chickpea (Table 3.9).

1998: Shoot dry weight data for the 1998 field season again showed that the granular inoculant treatments were significantly better at enhancing shoot dry weight as compared to the seed-applied inoculants in the desi (Tables 3.11 and 3.13), but not in the kabuli (Tables 3.12 and 3.14). Contrast analysis also indicated that placing the granular inoculant 8 cm below the seed resulted in higher shoot dry weight compared to 2.5-cm below seed placement in the desi at the early pod-filling stage (Tables 3.11 and 3.13). In both chickpea types, the peat-based inoculants were superior to the liquid inoculants in enhancing shoot dry matter (Tables 3.11-3.14).

As observed in 1997, the location x inoculation interaction was not significant for shoot dry matter in either the desi or the kabuli type at the early pod-filling stage, indicating that the inoculants performed similarly across locations (Tables 3.13 and 3.14). In general, in 1998 shoot dry weight of both desi and kabuli chickpeas at Watrous were higher than those at Outlook at the early pod-filling stage (Appendices 16-19). For example, the mean shoot dry weight for the kabuli chickpea at Watrous was 54% higher than the mean for the kabuli chickpea at Outlook (Appendices 18 and 19).

In 1998, shoot dry weight at the late pod-filling stage was evaluated only at Watrous. For the desi, dry matter at this stage for the non-inoculated control was not significantly different from those for the liquid inoculants and peat-based inoculant B (Appendix 14). As in the desi experiments, shoot dry matter in the kabuli experiments was lower in the liquid inoculant treatments than all the other inoculant treatments (Appendix 15). Although shoot dry weight was higher for the granular inoculants compared to the peat-based inoculants, they did not differ statistically.

3.3.2 Plot data

3.3.2.1 Biomass and seed yield

1997: At final harvest in 1997, plant biomass and seed yield for both kabuli and desi types, averaged over locations, were significantly increased by inoculation (Table 3.15 and 3.16, respectively). In particular, granular inoculant placed below the seed and seed inoculated with peat-based inoculant A produced the highest yields (Tables 3.17 and 3.18). The differences in plant biomass and seed yield between granular inoculants placed in the seed furrow and placement below the seed were significant for both kabuli (Table 3.15) and desi (Table 3.16), except for the seed yield in kabuli (Table 3.15). In each instance, granular inoculant below the seed performed better than granular inoculant placed with the seed.

The significant location by inoculation interaction for plant biomass and seed yield in the desi experiments (Table 3.16), was due primarily to the relative lack of response to inoculation at Elbow (Appendix 20) and Outlook (Appendix 21) as compared to the excellent response at Watrous and Kenaston (Appendices 23 and 25,

Table 3.15. Mean squares from the analysis of variance for whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea averaged over Kenaston and Watrous locations, 1997.

Sources of variation†	d.f.	Mean squares				
		Biomass	Seed yield	Protein conc.	%Ndfa	N_2 fixed
Locations (L)	1	1385018	934828	14.37	11260**	479
Reps in locations	6	1039076**	374000**	5.14**	625**	314**
Inoculation (I)	10	936536*	294595*	18.57**	1255 **	493**
Non-inoc vs. inoc	1	3160804**	1202501**	93.26**	8457**	2326**
Liq vs. peat	1	735078	229503	34.83**	1193*	648*
Liq A vs. liq C	1	65025	45156	0.07	1028*	151
Liq vs. gran	1	2752813**	809901**	76.23**	2732**	1831**
Peat A vs. peat B	1	636006	211600	7.49*	103	98
Peat vs. gran	1	371008	98102	2.26	99	135
Liq+peat vs. gran	1	2058010**	588700*	41.89**	1548**	1184**
Gran ws vs. gran bs	1	1776704*	344401	0.11	2	191
Gran 2.5 vs. gran 8.0	1	35113	528	0.37	85	26
Gran A vs. gran B	1	618802	288300	4.97	42	280
Gran str x ws vs. bs	1	22817	13301	0.11	215	71
Gran str x 2.5 vs. 8.0	1	37812	378	0.08	7	12
L x I	10	236088	75593	1.51	146*	97**
Error	60	142863	43071	1.42	67	38
Total	87					

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 3.16. Mean squares from the analysis of variance for whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea averaged over Elbow, Kenaston, Outlook and Watrous locations, 1997.

Sources of variation[†]	d.f.	Mean squares				
		Biomass	Seed yield	Protein conc.	%Ndfa	N_2 fixed
Locations (L)	3	56830089**	17182147**	79.21**	11120**	13561**
Reps in locations	12	1152347**	489958**	12.65*	1505**	394**
Inoculation (I)	10	919034**	357938**	17.38**	1622**	769**
Non-inoc vs. inoc	1	5090126**	1918621 **	84.21 **	8255**	3554**
Liq vs. peat	1	606452	336400	15.67*	4035**	1324**
Liq A vs. liq C	1	78	378	2.28	378	40
Liq vs. gran	1	1017846	441459	52.47**	4856**	2086**
Peat A vs. peat B	1	90313	29403	0.88	140	3
Peat vs. gran	1	3038	2109	5.73	66	1
Liq+peat vs. gran	1	452836	153015	59.73**	2744**	876**
Gran ws vs. gran bs	1	2671992**	1026675**	17.64*	1512**	1817**
Gran 2.5 vs. gran 8.0	1	205889	78400	9.93	7	8
Gran A vs. gran B	1	15504	14259	0.00	320	11
Gran str x ws vs. bs	1	3763	102	1.29	201	34
Gran str x 2.5 vs. 8.0	1	11827	1806	4.89	11	39
L x I	30	325172 *	115505*	3.67	191	112
Error	120	206927	70869	6.24	127	101
Total	175					

*, ** Significant at the 0.05 and 0.01 levels, respectively.

[†] Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

respectively). For both kabuli and desi experiments, biomass and seed yields were higher at Watrous (Appendices 22 and 23) than the other sites (Appendices 20, 21, 24 and 25).

Table 3.17. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea, averaged over Kenaston and Watrous locations, 1997.

Inoculant†	Biomass ($kg\ ha^{-1}$)	Seed yield ($kg\ ha^{-1}$)	Protein conc. ($g\ kg^{-1}$)	%Ndfa	N_2 fixed ($kg\ ha^{-1}$)
Non-inoc	1563	658	173	30.3	5.5
Liq A	1810	821	189	44.9	11.1
Liq C	1938	928	190	60.9	17.2
Peat A	2376	1159	217	62.6	25.6
Peat B	1978	929	203	67.6	20.7
Gran A with seed	2094	1054	214	70.6	25.3
Gran A 2.5 cm bs	2720	1290	220	70.2	32.2
Gran A 8.0 cm bs	2585	1291	219	66.0	29.2
Gran B with seed	2068	975	214	64.8	22.0
Gran B 2.5 cm bs	2324	1090	212	69.3	25.4
Gran B 8.0 cm bs	2326	1105	209	67.0	24.8
$LSD_{(0.05)}$	541	306	14	13.5	11.0
Contrasts‡					
Non-inoc vs. inoc	659**	406**	36**	34.1**	17.9**
Liq vs. peat	303	170	21**	12.2*	9.0 *
Liq A vs. liq C	128	107	1	16.0*	6.1
Liq vs. gran	479**	260**	25 **	15.1**	12.3 **
Peat A vs. peat B	398	230	14*	5.0	4.9
Peat vs. gran	176	90	5	2.9	3.3
Liq+peat vs. gran	327**	175*	15**	9.0**	7.8**
Gran ws vs. gran bs	408*	180	1	0.4	4.3
Gran 2.5 vs. gran 8.0	67	8	2	3.3	1.8
Gran A vs. gran B	227	155	6	1.9	4.8
Gran str x ws vs. bs	53	21	1	4.1	2.3
Gran str x 2.5 vs. 8.0	69	7	1	1.0	1.2

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

‡ Differences between specified treatments.

Table 3.18. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea, averaged over Elbow, Kenaston, Outlook and Watrous locations, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoc	1757	962	176	32.8	10.8
Liq A	2184	1211	187	41.4	17.9
Liq C	2188	1218	192	48.3	20.1
Peat A	2434	1390	197	58.7	27.8
Peat B	2328	1329	201	62.9	28.4
Gran A with seed	2199	1237	196	50.8	21.3
Gran A 2.5 cm bs	2437	1385	215	60.6	31.7
Gran A 8.0 cm bs	2578	1466	201	60.5	30.9
Gran B with seed	2113	1171	199	56.2	23.1
Gran B 2.5 cm bs	2469	1392	207	62.5	30.3
Gran B 8.0 cm bs	2556	1451	205	64.0	32.6
$LSD_{(0.05)}$	411	246	16	10.0	7.6
Contrasts‡					
Non-inoc vs inoc	592**	363**	24**	23.8**	15.6**
Liq vs. peat	195	145	10*	16.0**	9.1**
Liq A vs. liq C	4	7	5	6.9	2.2
Liq vs. gran	206	136	14**	14.3**	9.3**
Peat A vs. peat B	106	61	4	4.2	0.6
Peat vs. gran	11	10	5	1.7	0.2
Liq+peat vs. gran	109	63	10**	6.3**	4.8**
Gran ws vs. gran bs	354**	210**	10*	8.4**	9.2**
Gran 2.5 vs. gran 8.0	114	70	8	0.7	0.8
Gran A vs. gran B	25	25	0	3.6	0.7
Gran str x ws vs. bs	47	25	4	2.3	1.1
Gran str x 2.5 vs. 8.0	27	11	6	0.8	1.6

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

‡ Differences between specified treatments.

1998: Averaged over locations, seed inoculation with peat and granular inoculants placed with the seed in the kabuli chickpea resulted in higher yields compared to the other treatments (Table 3.19). However, the contrast of liquid or peat-based inoculant vs. granular inoculant was not significant for either biomass or seed yields. Biomass and seed yields in 1998 at the Outlook were affected by droughty conditions (Appendix 1), and the effect was most severe in the treatments where the inoculants were placed below the seed in the kabuli experiment due to problems encountered with seed placement, as previously described. With granular inoculation, e.g., granular inoculant B placed 8 cm below the seed was the only treatment that reduced biomass and seed yield of kabuli significantly below the non-inoculated control treatment (Appendix 26). Inoculation did not affect biomass and seed yield at Watrous, except for the biomass yield enhancement due to granular A placed with the seed (Appendix 27). Unlike the 1997 experiments and the desi experiments in 1998, biomass for the kabuli in 1998 was significantly higher (P = 0.03) in granular A than granular B inoculants, although the difference in seed yield was not significant (Table 3.19).

Desi biomass and seed yields averaged over locations were significantly higher in the inoculation treatments than in the control (Table 3.20). On average, inoculating the soil with granular inoculants consistently increased biomass and seed yields over that for seed-applied liquid inoculant in the desi, but the contrast of peat vs. granular indicated no significant difference. The peat inoculation resulted in higher biomass and seed yields than liquid inoculation. At Outlook, desi biomass and seed yields were significantly increased by inoculation (Appendix 28), but unlike Watrous (Appendix 29), both biomass and seed yields for granular inoculants placed 8 cm below seed were lower than the other granular inoculant treatments and the peat inoculants, as was reported above for kabuli. At Watrous, the maximum biomass yield was obtained with granular A inoculant placed in the seed furrow at planting and was 1659 kg ha^{-1} over the control. Similarly, the greatest increase in seed yield due to inoculation was 644 kg ha^{-1} and occurred in granular B placed 8 cm below the seed. Despite the apparent differences in the kabuli experiments, no significant location x inoculation interactions were observed for biomass and seed yields in either chickpea type in 1998, although

location had significant effect on both parameters in desi and kabuli chickpeas (Tables 3.21 and 3.22).

Table 3.19. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea averaged over Outlook and Watrous locations, 1998.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoc	3742	1218	190	7.5	3.3
Liq A	3734	1251	207	21.1	11.3
Liq B	3717	1246	207	28.5	14.0
Peat A	3940	1317	239	40.6	20.4
Peat B	3958	1393	233	41.7	22.0
Gran A with seed	4361	1362	243	40.7	21.7
Gran A 2.5 cm bs	4190	1268	246	44.2	22.5
Gran A 8.0 cm bs	4062	1176	243	37.3	17.1
Gran B with seed	4062	1360	230	44.1	22.5
Gran B 2.5 cm bs	3869	1185	243	39.4	18.7
Gran B 8.0 cm bs	3487	954	258	30.3	14.0
$LSD_{(0.05)}$	613	285	42	16.1	7.2
Contrasts‡					
Non-inoc vs inoc	196	33	45**	29.3**	15.1 **
Liq vs. peat	224	107	29	16.4**	8.6**
Liq A vs. liq B	17	5	0	7.4	2.7
Liq vs. gran	280	31	37**	14.5**	6.8**
Peat A vs. peat B	18	76	6	1.1	1.6
Peat vs. gran	56	137	8	1.8	1.8
Liq+peat vs. gran	168	84	22*	6.4	1.5
Gran ws vs. gran bs	310	215*	11	4.6	4.0
Gran 2.5 vs. gran 8.0	255	162	6	8	5.1*
Gran A vs. gran B	398*	102	0	2.8	2.0
Gran str x ws vs. bs	270	108	0	4.2	3.1
Gran str x 2.5 vs. 8.0	127	70	9	1.1	0.4

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

‡ Differences between specified treatments.

Table 3.20. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea averaged over Outlook and Watrous locations, 1998.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoc	2311	1222	156	17.2	6.7
Liq A	3056	1521	169	19.9	9.8
Liq B	2971	1495	174	44.0	20.9
Peat A	3504	1813	193	49.1	27.5
Peat B	3564	1751	217	53.7	34.7
Gran A with seed	3733	1805	200	56.9	34.0
Gran A 2.5 cm bs	3571	1748	237	60.3	39.6
Gran A 8.0 cm bs	3418	1690	230	59.3	36.8
Gran B with seed	3503	1755	208	54.9	33.7
Gran B 2.5 cm bs	3459	1731	222	54.6	34.1
Gran B 8.0 cm bs	3418	1726	226	53.5	35.2
$LSD_{(0.05)}$	490	210	32	14.7	10.7
Contrasts‡					
Non-inoc vs. inoc	1109**	482**	52**	33.4**	24.0**
Liq vs. peat	521**	274**	34**	19.5**	15.8**
Liq A vs. liq B	85	26	5	24.1**	11.1*
Liq vs. gran	504**	235**	50**	24.6**	20.2**
Peat A vs. peat B	60	62	24	4.6	7.2
Peat vs. gran	17	39	16	5.2	4.5
Liq+peat vs. gran	243*	98*	32**	14.9**	12.3**
Gran ws vs. gran bs	152	56	25*	1.0	2.8
Gran 2.5 vs. gran 8.0	97	32	2	1.1	0.9
Gran A vs. gran B	114	10	4	4.5	2.5
Gran str x ws vs. bs	141	38	2	1.4	0.7
Gran str x 2.5 vs. 8.0	56	27	6	0.1	2.0

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

‡ Differences between specified treatments.

Table 3.21. Mean squares from the analysis of variance for whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea averaged over Outlook and Watrous locations, 1998.

		Mean squares				
Sources of variation†	d.f.	Biomass	Seed yield	Protein conc.	%Ndfa	N_2 fixed
Locations (L)	1	246761018**	13612742**	51.98	1555*	4733**
Reps in locations	6	1040102**	57615	3.39	151**	79**
Inoculation (I)	10	485540	118359	34.33*	1045**	289**
Non-inoc vs. inoc	1	278357	7789	148.11**	6222**	1672**
Liq vs. peat	1	401184	90525	65.14	2140**	579**
Liq A vs. liq B	1	1139	110	0.01	220	30
Liq vs. gran	1	939820	11625	160.96**	2546**	544**
Peat A vs. peat B	1	1296	22952	1.57	5	10
Peat vs. gran	1	37520	226875	7.85	38	38
Liq+peat vs. gran	1	541162	136485	95.97*	784	118
Gran ws vs. gran bs	1	1019907	494214*	13.07	225	170
Gran 2.5 vs. gran 8.0	1	520455	208335	3.38	514	207*
Gran A vs. gran B	1	1903237*	124033	0.05	93	50
Gran str x ws vs. bs	1	818258	70525	1.22	55	49
Gran str x 2.5 vs. 8.0	1	129413	38642	6.46	9	1
L x I	10	303212	65225	14.35**	209**	41*
Error	60	334034	334034	3.38	44	19
Total	87					

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

Table 3.22. Mean squares from the analysis of variance for whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea averaged over Outlook and Watrous locations, 1998.

Sources of variation†	d.f.	Mean squares				
		Biomass	Seed yield	Protein conc.	%Ndfa	N_2 fixed
Locations (L)	1	92194833**	10857137**	252.86**	1135*	7529**
Reps in locations	6	516785**	146772**	5.19	64	71
Inoculation (I)	10	1234088**	254555**	58.79**	1814**	996**
Non-inoc vs. inoc	1	8414125**	1688052 **	189.62 **	8134**	4150**
Liq vs. peat	1	2164240**	598965**	89.93**	3033**	1980**
Liq A vs. liq B	1	28561	2730	0.97	2326**	495*
Liq vs. gran	1	3057018**	659063**	293.90**	7281**	4849**
Peat A vs. peat B	1	14280	15068	22.75	84	203
Peat vs. gran	1	2844	18506	30.57	319	239
Liq+peat vs. gran	1	1149346*	182676*	205.62**	4260**	2920**
Gran ws vs. gran bs	1	237407	33675	66.78*	11	70
Gran 2.5 vs. gran 8.0	1	69938	8065	0.11	9	6
Gran A vs. gran B	1	164385	1302	1.75	243	77
Gran str x ws vs. bs	1	279720	17821	1.07	26	4
Gran str x 2.5 vs. 8.0	1	21528	5618	2.64	0	31
L x I	10	193441	35706	8.47**	174*	91
Error	60	152242	43289	3.15	82	48
Total	87					

*, ** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated, Liq = liquid, Gran = granular, ws = with seed, bs = below seed, str = strain.

3.3.2.2 Seed protein concentration, percentage N derived from atmosphere (%Ndfa) for the seed, and amount of seed N derived from fixation

1997: Seed protein and proportion and amount of N_2 fixed for the seed averaged over locations in 1997 were significantly higher for inoculated plants in both chickpea types (Tables 3.15-3.18). The highest protein concentration, %Ndfa and amount of N_2 fixed generally occurred for soil inoculation treatments, particularly granular inoculant A placed 2.5 cm below the seed but contrasts of peat vs. granular indicated no significance (Tables 3.15-3.18). For both kabuli and desi chickpeas, seed inoculated with liquid inoculants on average, resulted in lower %Ndfa, amount of N_2 fixed and seed protein concentration than the average for the peat or granular inoculant treatment. However, except for liquid A, the %Ndfa associated with liquid C statistically was not different from all the other inoculation treatments in the kabuli (Table 3.17). In contrast to the general trend, seed inoculation with peat A produced significantly higher seed protein concentration than that for peat B in the kabuli chickpea. For the desi chickpea, placing the granular inoculants below the seed significantly increased %Ndfa, N_2 fixed and seed protein concentration compared to placement in the seed furrow (Table 3.18). As was the case for the other yield parameters, no differences in *Rhizobium* strain were observed in either chickpea type.

In the kabuli experiments, a significant location x inoculation interactions were observed for %Ndfa and N_2 fixed (Table 3.15), due primarily to the low N_2 fixation for the granular B inoculant relative to the granular A inoculant at Watrous (Appendix 22) and the higher %Ndfa for the peat B inoculant at Kenaston (Appendix 24). A significant location effect was also found for %Ndfa and amount of N_2 fixed in the kabuli chickpea as a result of the higher fixation at Kenaston (Appendix 24) than Watrous (Appendix 22). Among the desi experiments, no significant location x inoculation interactions for protein concentration, %Ndfa or N_2 fixed was observed (Table 3.16). However, location had a significant effect on protein concentration, %Ndfa and N_2 fixed, due primarily to the higher seed protein concentration at Outlook as compared to the other locations and the higher N_2 fixation at Kenaston and Watrous relative to that at Elbow and Outlook (Appendices 20, 21, 23 and 25).

1998: In 1998, %Ndfa, amount of N_2 fixed and seed protein concentration for both chickpea types followed a similar trend as observed in the 1997 experiments (Tables 3.19 and 3.20). However, whereas the average %Ndfa was lower in 1998, the average seed protein concentration was higher in 1998 than 1997. Contrasts of peat vs. granular indicated no significant differences in %Ndfa, N_2 fixed and seed protein concentration. As in the other parameters measured, the liquid inoculants were inferior to the other inoculation treatments in %Ndfa, amount of N_2 fixed and seed protein concentration.

In contrast to the 1997 experiments, significant interactions between location and inoculation were found for %Ndfa, N_2 fixed and seed protein concentration in both kabuli and desi chickpeas, except for the N_2 fixed in the desi (Tables 3.21 and 3.22). However, the seed protein concentration at Outlook for the granular inoculant B placed below the seed was exceptionally high (Appendix 26). In the kabuli chickpea, %Ndfa for the granular inoculant placed below the seed was generally low at Outlook as compared to that at Watrous (Appendices 26 and 27). The amounts of N_2 fixed in all treatments were also lower at Outlook than at Watrous. For the seed protein concentration, the values for the non-inoculated and the liquid inoculant treatments at Watrous were considerably higher than those obtained at Outlook.

In the desi chickpea, seed protein concentration, %Ndfa and the amount of N_2 fixed were higher at Watrous as compared to Outlook (Appendices 28 and 29). The significant location by inoculation interaction for protein concentration was due primarily to the low values for granular B treatment at Outlook (Appendices 28) relative those at Watrous (Appendix 29). The significant location by inoculation interaction for the %Ndfa was due primarily to the low %Ndfa values for the non-inoculation control and liquid A at Outlook (Appendix 28) relative to those for Watrous (Appendix 29).

3.3.2.3 Correlations between crown or lateral root nodules and shoot dry matter production and seed yield

1997: In both chickpea types averaged over locations, the dry weight of lateral root nodules on an individual plant basis was highly positively correlated with plant dry

matter production at the flowering (not shown), early pod-filling and late pod-filling stages (Table 3.23). In contrast, the correlation between the dry weight of crown nodules and shoot dry matter production was weak and not significant. Similarly, seed yield was highly correlated with the dry weight of lateral root nodules, whereas little or no correlation existed between dry weight of the crown nodules and seed yield.

Table 3.23. Correlations between the dry weight of lateral or crown nodules at early and late pod-filling stages and seed yield, and shoot dry matter.

Character†	----------Desi----------	--------Kabuli--------
	r	r
	Early pod-filling stage, 1997	
Shoot DM and lateral nodules	0.88**	0.92**
Shoot DM and crown nodules	0.24	0.22
Seed yield and lateral nodules	0.69*	0.73**
Seed yield and crown nodules	0.00	0.10
	Late pod-filling stage, 1997	
Shoot DM and lateral nodules	ND‡	0.82**
Shoot DM and crown nodules	ND	0.26
Yield and lateral nodules	ND	0.80**
Yield and crown nodules	ND	0.10
	Early pod-filling stage, 1998	
Shoot DM and lateral nodules	0.91**	0.66*
Shoot DM and crown nodules	0.14	0.33
Yield and lateral nodules	0.66*	0.17
Yield and crown nodules	0.48	0.66*

*, ** Significant at the 0.05 and 0.01 levels, respectively.
†DM = dry matter
‡ND = not determined.

1998: Similar to the 1997 results, shoot dry matter and the dry weight of lateral root nodules at early pod-filling was highly correlated but the correlation between shoot dry matter and dry weight of crown nodules was not significant in either chickpea type (Table 3.23). However, unlike 1997, seed yield in the kabuli chickpea was weakly correlated with the dry weight of lateral root nodules at the early pod-filling stage, due primarily to the delayed germination and reduced plant stand as result of the severe drought. In this case, the correlation between seed yield and the dry weight of crown nodules at early pod-filling was significant. In the desi chickpea, dry weight of lateral root nodules at the early pod-filling stage was positively correlated with seed yield, but dry weight of crown nodules was not correlated with seed yield.

3.4 Discussion

Initially, the experiments were planned only for desi chickpea, but it was later decided to include kabuli chickpea in separate experiments. Because direct comparison between the two genotypes was not of major interest, separate experiments were conducted for each genotype. Nevertheless, the two genotypes responded similarly to the method of inoculation and the rhizobial strain combinations. Although reports on chickpea by Corbin et al. (1977) and Chandra and Pareek (1985) indicated no interaction between strains of rhizobia and genotype, Somasegaran et al. (1988) demonstrated that, under certain soil conditions and with the use of mixed inoculant strains, a significant strain x genotype interaction can occur. The inoculant obtained from MicroBio RhizoGen Corp., designated A, and Agruim Biologicals, designated C, consisted of single strains, whereas that obtained from LiphaTec, designated B, was a mixture of three strains. However, strains in similar formulations performed equally throughout the experiments and nothing indicated any differential response to rhizobial strains or between chickpea types in any of the parameters measured.

Following inoculation and seeding, the course of rhizosphere and root colonization by inoculant strain and subsequent nodule formation and N_2 fixation followed a predicted sequence (Brockwell et al., 1985; Herridge et al., 1988), subject to environmental conditions (Alexander, 1985). In these experiments, seeds were sown

at moderate air temperatures and into good moisture, except at Elbow in 1997 and Outlook in 1998, where the available soil moisture levels were low. Hence, adequate available soil moisture favoured the establishment of a successful symbiosis. Nevertheless, a decline in rhizobia numbers might have occurred in the liquid inoculant treatments since nodulation was generally lower than that for the peat and granular inoculants. Peat, when used as inoculant carrier, protects rhizobia inoculated onto the seed to some extent from desiccation (Hansen, 1994). Hansen (1994) indicated that when rhizobia in liquid inoculant are inoculated onto the seed, they are relatively more susceptible to unfavourable environmental conditions, such as desiccation and excessive heat during and after seeding. Roughley et al. (1993) demonstrated the effect of desiccation on rhizobia inoculated onto seed when 95% of the rhizobia originally present in the inoculant died during inoculation and sowing, and a further 85% of the remaining rhizobia lost viability during the following day in the soil. Death of rhizobia on seed between inoculation and seeding due to high temperature was also reported by Brockwell et al. (1987). Although temperatures were not high during seeding, it was generally dry and windy, and survival of the rhizobia may have been affected by desiccation, contributing to the low nodulation in the liquid inoculant treatments as compared to the other inoculation treatments.

The seeds were treated with fungicides (Apron and Crown) before inoculation and these treatments may have contributed to the low nodulation from the liquid inoculant by decreasing rhizobia survival. Several studies have shown that some seed–applied fungicides are incompatible with rhizobia (Ramos and Ribeiro, 1993; Revellin et al., 1993). However, compared to liquid formulation a peat formulation may help protect rhizobial strains to some extent from antagonistic components that would reduce their populations (Zdor and Pueppke, 1990). In greenhouse and field studies, Revellin et al. (1993) observed decreased survival of *B. japonocum* and reduced nodulation and yield of soybean when Apron was used as a seed treatment. The extremely poor nodulation in the liquid treatments at Outlook in 1998 (Appendices 16 and 19) likely was the result of severe drought during and after seeding. On the other hand, the soil moisture status at Watrous in 1997 was relatively good; hence,

nodulation in the liquid inoculant treatment was comparable to that of the other treatments (Appendices 4 and 8).

Nodule numbers in the peat and granular inoculant treatments were not consistent over the years (Tables 3.3, 3.4, 3.7, 3.8, 3.11 and 3.12). In 1997 the peat inoculants produced more nodules than the granular inoculants, but in 1998 the granular inoculants formed more nodules than the peat inoculants. No consistent relationship between total nodule dry weight and total nodule numbers existed. Despite the higher total nodule numbers in the peat inoculant treatments in 1997, the total dry weights were similar to those for the granular inoculants. This agrees with previous observations by Smith et al. (1981) and Danso and Bowen (1989), who reported that, when soybean plants have only a few nodules on their roots, the nodules usually grow much larger than on plants which have many nodules.

In contrast to the results observed in 1997, the dry weights of the nodules for the peat-based inoculants in 1998 were generally greater than those for the granular inoculants. This observation was, however, more pronounced at Outlook than Watrous. Planting at Outlook in 1998 was on 20 May, when the soil was dry, as mean monthly precipitation was 57% less than normal (Appendix 1). As a result of the hard soil surface, the additional opener for the granular inoculant placed below the seed increased resistance of the soil to penetration, resulting in deposition of the seeds just below the soil surface, into an area too dry for optimum germination and emergence. Therefore, seed germination in treatments with granular inoculants below the seed did not occur until after a rain and well after seeding. Hence, at the time of sampling, nodule formation and development in these treatments were a little behind that in the peat treatments and the treatment with granular inoculant placed at the seeding depth. This likely accounted for the lower dry weight of the treatments with granules below the seed.

Nodulation by the inoculant rhizobia, following delayed germination and growth of the chickpea seedlings, suggests that the strains survived well under the drought conditions. This is consistent with the report by Brockwell et al. (1980), who stated that when conditions are unfavourable for rhizobial survival, or when germination is delayed due to adverse environmental conditions, soil inoculation

produced better nodulation than seed-applied inoculation. The kabuli chickpea was more affected by the drought than the desi because its larger seed requires more moisture for germination and nodulation. Therefore, nodulation in the kabuli chickpea was lower at Outlook than Watrous in 1998 and, for this reason a significant site x inoculation interaction was observed for total nodule numbers and dry weight.

Although limited, some nodulation was observed on the non-inoculated plants. This unexpected nodulation likely was due to low levels of plot-to-plot contamination. Growth chamber studies prior to seeding indicated that the soil from all experimental sites contained no native chickpea rhizobia. Moreover, a nine-year cropping history of the sites indicated no legume or chickpea cultivation. Kamicker and Brill (1987) observed that some rhizobial strains introduced to soil can persist for many years and are capable of nodulating subsequent crops. Even if a legume crop had been grown on any of these sites in the recent past, it is not likely the resident rhizobia could nodulate the chickpea plants due to their highly specific rhizobial requirements (Gaur and Sen, 1979; Silsbury, 1989). However, nodulation in the control treatments was sparse compared to the inoculated treatments and had no significant effect on the results.

In agreement with other researchers (Smith et al., 1981; Danso and Bowen, 1989; Kahn and Stoffella, 1991), the dry weight of nodules was considered a more accurate measure of N_2-fixing potential than nodule numbers, due to the wide variation in nodule size. The location of nodule formation on the root system varied, depending on the inoculation method. Seed inoculation produced nodules predominantly at the crown region of the root, whereas soil inoculation resulted in most of the nodules in the lower part of the root system, i.e., on the lateral roots. Inoculation with granular inoculant at the seeding depth resulted in substantial nodulation on the lateral roots, but this proportion increased as the granular inoculant was placed below the seed. The nodulation pattern observed in this study is consistent with data from other studies in which deeper placement of rhizobial inoculants in the soil resulted in substantial nodule formation below the topmost 10 cm region of the root (Wilson, 1975; Wadisirisuk et al., 1989).

Danso and Bowen (1989) observed that nodule formation was restricted to the vicinity of the point of inoculum placement. In soybean, they reported that, seed

inoculation resulted in 94% of the nodules on the tap root and on the roots 0-5 cm from the stem base, whereas soil inoculation resulted in a lower proportion (i.e., 63%) of the nodules in this zone. In another study, Hardarson et al. (1989) also reported that seed inoculation resulted in formation of 87% of the nodules on the tap roots 0-5 cm below the base of the stem, whereas soil inoculation resulted in only 20-40% of the nodules in this zone. In the present study, less than 40% of the nodules in the seed-inoculated plants was located on the lateral roots, whereas as much as 97% of the nodules in the granular inoculant placed below the seed was formed on the lateral roots. Kamicker and Brill (1987) observed that inoculant in the seed furrow produced nodules mainly in the upper region of soybean root system, whereas inoculant tilled into the soil produced nodules primarily in the lower region of the root system.

The preponderance of nodulation in the root zone immediately below the position of inoculum placement indicates limited migration by the rhizobia. According to Madsen and Alexander (1982), *B. japonicum* did not move more than 2.7 cm in the absence of infiltrating water. Hence, distribution of nodules on the entire root system requires that the roots encounter the inoculant rhizobia in the soil and this may require relatively large populations (Zablotowicz et al., 1991). The higher number of lateral root nodules from placement of the granular inoculant in the seed furrow, compared to the seed-applied inoculants, further indicates the poor mobility of rhizobia in the soil. Early formed crown nodules generally suppress further nodule formation on the younger roots (Kosslak and Bohlool, 1984; George et al., 1992). However, the considerable nodulation at the crown region and on the lateral roots in the treatments with granular inoculant placed with the seed suggests that any suppression by early-formed nodules may be partial. Rather, the crown nodulation pattern with seed inoculation was due largely to the limited migration of the rhizobia to other infectible sites along the root. Caetano-Anolles et al. (1992) attributed the crown nodulation pattern to the inability of the rhizobia to move with the developing root system.

Higher shoot dry matter was produced at all sampling dates when granular inoculants were used as compared to the peat and liquid inoculants. However, the liquid was again inferior to the peat treatment in shoot dry matter production. Higher production of dry matter yields with granular inoculants has been reported in alfalfa

(Rice and Olsen, 1988, 1992) and arrowleaf clover (Ocumpaugh and Smith. 1991). In contrast, Hardarson et al. (1989) found that the higher N_2 fixation following soil inoculation did not translate into increased plant dry matter yield as N was not a limiting factor in the soil used. Inoculation had little effect on shoot dry matter at either the flowering or the early pod-filling stage in desi chickpea at Elbow in 1997. However, the low available soil moisture level at Elbow, compared to the other sites (Table 3.1), likely limited response to some of the individual inoculant treatments.

Sprent (1972) and Durand et al. (1987) demonstrated the importance of adequate available soil moisture for maximum N_2 fixation by grain legumes. Furthermore, Zapata et al. (1987) showed that the highest rates of N_2 fixation in field-grown soybean occurred during the periods of active sink development. This was later confirmed by Danso et al. (1990), who showed that the highest rate of N accumulation from N_2 fixation occurred between early pod development and physiological maturity in soybean. Dinitrogen fixation was not assessed in chickpea during these sampling dates, but it is possible that N_2 fixation at the flowering and early pod-filling stages at Elbow was not high enough to cause significant differences in shoot dry matter among the inoculant treatments. This explanation is supported by the finding that shoot dry weight was higher for soil inoculation than seed inoculation at the 5% level of probability at flowering in desi chickpea, but at the early pod-filling, this difference increased and was significant at the 1% level (Tables 3.3 and 3.4). Perhaps, plants with lateral root nodules were delayed in the onset of N_2 fixation because the nodules were formed relatively late and not fully developed by the flowering stage. However, at Outlook in 1998, the delayed emergence of the treatments with granular inoculants below the seed had little effect on dry matter production as dry matter yield per plant was in general higher for soil inoculation than seed inoculation. The lower dry matter production with the liquid inoculant treatment, relative to the non-inoculated control, could not be adequately accounted for because in most cases the desi and kabuli chickpea plants nodulated at both Outlook and Watrous.

The value of inoculation was demonstrated in seed yield and plant biomass at final harvest. Although, the yield increases in 1997 were not consistently significant among inoculation treatments and across locations in both types of chickpea, granular

inoculants placed below the seed were superior to the other inoculant treatments. In 1997, the maximum increase in seed yield averaged over locations in the kabuli chickpea was 633 kg ha^{-1} and occurred when granular inoculant A was placed below the seed followed by granular inoculant B placed below the seed (440 kg ha^{-1}) (Table 3.17). Seed yield differences between the peat and granular inoculants were relatively low and insignificant. For example, for the kabuli chickpea, the average seed yield increases for granular inoculant below the seed were 151 (14%) and 320 kg ha^{-1} (36%) greater than for the peat and liquid inoculants, respectively (Table 3.17). For the desi chickpea, seed yield increases for the granular inoculants placed below the seed were 64 kg ha^{-1} and 209 kg ha^{-1} (5 and 17%, respectively) greater than for the peat and liquid inoculants, respectively (Table 3.18). The limited yield increases associated with granular inoculant below the seed may be due, in part, to better moisture conditions in this soil zone and extra protection from heat for the rhizobia and, subsequently, for the nodules, favouring N_2 fixation. The formation of nodules later in the growing season and the greater duration of N_2 fixation in these treatments also may have contributed to the higher yields. The plants inoculated with granular inoculant placed in the furrow with the seed were nodulated adequately both at the crown and the lower part of the root system, and the proportion and amount of N_2 fixed were similar or higher than those for the seed-applied inoculants. Hence, the cause of the generally lower seed yield of this treatment as compared to the seed-applied inoculants, in particular the peat-based could not be adequately explained.

In other studies, yield increases of 38% (Scudder, 1975), 60% (Bezdicek et al., 1978) and 20% (Muldoon et al., 1980) were reported in soybean for granular inoculant over seed-applied inoculant. Dean and Clark (1977) also reported a seed yield increase of 730 kg ha^{-1} over seed-applied inoculant, when granular inoculant was used in a study with faba bean. In the present study, the yield advantage for the granular inoculant was low, compared to that reported for soybean and faba bean. The reason for the limited yield increase may be related to the inoculation rate used. The beneficial effect of massive inoculation is well documented (Weaver and Frederick, 1974a, b; Thies et al., 1991; Roughley et al., 1993) and one of the major advantages of soil inoculation is that the rhizobial application rate can be increased far beyond that

applied by seed inoculation. Granular inoculants were used at the recommended rate in the present study, whereas rates higher than recommended were used by other workers (e.g., Bezdicek et al., 1978; Muldoon et al., 1980). For example, Muldoon et al. (1980) used three times the recommended rate of granular inoculant, whereas Bezdicek et al. (1978) used twice the recommended rate, although the latter observed limited yield increase when the recommended rate of inoculant was used.

Inoculating legume crops at such a high rate may not be economical considering the higher cost of the granular inoculants as compared to the seed-applied inoculants. The results of the 1997 study indicate that the extra cost for the granular inoculant was more than recovered for the granular inoculant placed below the seed in the kabuli. However, in the desi the value of the yield increase was slightly more than the additional cost of the granular inoculant. The results also indicate that soil inoculation at the seeding depth was not economical.

In 1998, only granular inoculant A placed in the seed furrow increased plant biomass significantly in kabuli chickpea at Watrous and inoculation had no significant effect on seed yield (Appendix 27). Available soil N was possibly not a limiting factor in this soil; hence, N_2 fixation was not translated into biomass or seed yield. Soils at Watrous had a relatively high organic matter content (4.1%) and, given adequate moisture, may exhibit high rates of mineralization that provided sufficient soil N available to the plants.

The dry soils reduced the seeding depth when granular inoculants were placed below the seed and resulted in uneven stands and delayed plant growth, precluding realistic yield results, particularly with kabuli chickpea at Outlook in 1998. Scudder (1975) obtained higher seed yield under dry soil conditions in Florida with granular inoculation than seed-applied inoculation. Brockwell et al. (1980) also stated that, when conditions were unfavourable for rhizobial survival, or when germination was delayed due to environmental conditions, soil inoculation was superior to seed inoculation. However, this will only apply if plant density is not affected and the length of time for active N_2 fixation is not shortened. At Outlook, the delayed germination drastically reduced the length of the available growing season and reduced plant density, resulting in seed yields from granular B inoculant placed below

the seed that were even lower than for the non-inoculated control (Appendix 26). Data on nodule dry weight and shoot dry matter per plant at the early pod-filling stage confirmed that plant growth was not affected despite the delayed germination. Hence, the lower seed yield was primarily due to the lower number of plants per ha and the short growing season. Chickpea is a long season crop as compared to other grain legumes, such as common bean and pea (Saskatchewan Pulse Crop Development Board, 1997). However, low precipitation in July (e.g., 32 % of normal at Elbow in 1997 and 11 and 39 % of normal in 1997 and 1998, respectively, at Outlook) and relatively high temperatures in August (Appendix 1) resulted in terminal drought which shortened the ripening period. Since the onset of N_2 fixation by nodules formed with granular inoculation may be delayed, these nodules could not fully express their N_2-fixing capacity under terminal drought. Thus, indeterminate cultivars of common bean typically have a longer growth cycle, fix more N and produce higher yields than determinate cultivars with a shorter growing season requirement (Rennie and Kemp, 1983, 1984; Vessey, 1992). Ciafardini and Lombardo (1991), using cover inoculation (liquid inoculum applied to the soil with irrigation water) of previously seed-inoculated soybean plants, found that the benefits of cover inoculation on yield and seed protein concentration may decrease when the growth period is shortened. This may be another possible explanation for the improved, but limited, performance of the granular inoculant placed below the seed.

Higher levels of seed protein concentration and seed N derived from the atmosphere were generally obtained with soil inoculation as compared to those for the seed inoculation with liquid inoculant and reflected the trend observed in plant biomass and seed yields. However, the differences in these traits between the soil inoculation and seed inoculation with peat-based inoculant were not significant, although numerically, they were higher for the former. Previous reports on soybean indicate that soil inoculation produced higher N_2 fixation (Muldoon et al., 1980; Dubetz et al., 1983; Hardarson et al., 1989) and seed protein concentration (Muldoon et al., 1980) as compared to seed inoculation. With increasing granular inoculant rate, higher concentrations of seed protein were obtained by Muldoon et al. (1980), indicating that poor nodulation and N_2 fixation limited protein production. In general,

the proportion of N_2 derived from fixation was higher in 1997 than 1998 whereas protein concentration was higher in 1998 than 1997, due to the more favourable growth conditions in 1997. This supports the negative correlation between yield and protein concentration, which often occurs in grain legumes under "normal" growing conditions (Williams and Nakkoul, 1983). Westerman et al. (1985) observed an association between low seed yield in bean and high nitrogen concentration. Apparently, the decrease in seed yield due to moisture stress in 1998 was greater relative to seed nitrogen yield and resulted in a higher protein concentration.

The results of the present study indicate that differences in yield parameters were likely influenced by the nodulation pattern rather than the number or dry weight of nodules. Several studies (e.g., Wolyn et al., 1989; Danso et al., 1990) have shown that the widely held opinion that dense nodulation at the crown region is evidence of successful inoculation and, thus, high N_2 fixation (Vincent, 1970) is inconsistent in soybean and bean. Rather, lateral root nodulation is important in N_2 fixation in soybean (Hardarson et al., 1989; McDermott and Graham, 1989) and common bean (Wolyn et al., 1989; Vikman and Vessey, 1992, 1993), particularly during the reproductive stage. Nodules at the crown region are the first to be formed and are active during the early growth stages of plant, but, according to Bergensen (1958), the activity of such nodules in soybean persists for an average duration of 65 days. Hence, nodules that develop later on the lateral roots may be essential since they remain active during the entire period of high N demand at pod-filling and seed maturation (Ciafardini and Barbieri, 1987; Zapata et al., 1987; Imsande, 1989).

Danso et al. (1990), using the ^{15}N isotope dilution technique, demonstrated that seed inoculation, which formed mostly crown nodules, fixed more N_2 than soil inoculation, which produced mainly lateral root nodules at the early pod-filling stage, but this trend was reversed at physiological maturity. Similarly, McDermott and Graham (1989) found that crown nodules accounted for 100% acetylene reduction activity 20 days after planting (DAP), but the activity declined to less than 20%, at 76 DAP (pod-fill), due to nodule senescence and the steady increase in nodule mass on the lateral roots. Therefore, granular inoculant permits the young lateral roots to come into direct contact with the inoculant for infection and nodule formation. Thus,

granular inoculant enhances lateral root nodulation, which can contribute significantly to N_2 fixation and yield. To test this hypothesis, shoot dry matter was correlated separately to dry weight of crown and lateral root nodules on an individual plant basis averaged over all locations. A significant positive correlation occurred between dry weight of the lateral root nodules at the early pod-filling and late pod-filling stages and shoot dry matter production or seed yield in 1997 (Table 3.23), indicating that increased lateral root nodulation was associated with high yields. Drought at Outlook during seeding in 1998 diminished the correlation between lateral root nodules and crop yield in kabuli chickpea, but for desi chickpea, the lateral root nodules was significantly correlated with shoot dry matter at early pod-filling and seed yield at maturity. Generally, correlation between the crown root nodules and these traits was low and not significant. The strong association between yield (shoot dry matter and seed) and lateral root nodules indicates that these nodules often determine, to a large extent, the yield of nodulated legumes. This is because these lateral root nodules were formed later and remain active during the reproductive phase and, thus, have a greater effect on yield than crown nodules.

The data highlight the need to improve the current method of inoculation to ensure sufficient nodulation of the lateral roots. Due to the limited migration of rhizobia in the soil, seed inoculation often results in crown root nodulation, and as these nodules approach senescence, the plant may be dependent on nodules formed by indigenous strains which may be less efficient in N_2 fixation (Vance and Graham, 1995). Soil inoculation below the seed is one possible way to enhance lateral root nodulation since the inoculum can be positioned in the soil zone to target the young developing roots. Howieson and Ewing (1986), working with *R. meliloti*, found some evidence of differential mobility among strains of rhizobia. Thus, nodulation away from the immediate vicinity of inoculum placement may be improved by using more motile inoculant strains (Ames and Bergman, 1981). Although differential responses to inoculation methods may be reduced in a year with optimum weather conditions, unfavourable growing conditions are often unavoidable during and after seeding. Thus, granular inoculant formulations, which protect the rhizobia from environmental stresses, may be superior to other inoculants in certain years. The ability to use higher

inoculum rates with soil inoculation than with seed inoculation suggests that greater yield increases in chickpea could accrue from using higher rates of granular inoculants rather than liquid or peat inoculants, especially in first-time chickpea fields.

4. ISOTOPIC FRACTIONATION DURING N_2 FIXATION AND CHICKPEA GROWTH

4.1 Introduction

Estimation of atmospheric N_2 fixation in plants by the ^{15}N natural abundance technique is based on the fact that the $^{15}N/^{14}N$ ratio of the soil is slightly higher than in atmospheric N_2 (Amarger et al., 1979; Kohl and Shearer, 1980, Shearer and Kohl, 1986; Danso et al., 1993). Thus, an N_2-fixing plant, which depends on both soil N and symbiotic N_2 fixation, would be less abundant in ^{15}N than a non-fixing plant grown at the same site (Rennie et al., 1976; Kohl and Shearer, 1980). This small, but measurable, difference in ^{15}N abundance between the symbiotic N_2-fixing and non-fixing plants has been used to quantify the contribution of atmospheric N_2 to the total N of the N_2-fixing plant (Bremer and van Kessel, 1990; Doughton et al., 1995; Herridge et al., 1995). Although the natural abundance of ^{15}N in the atmospheric N_2 is constant (Mariotti, 1983), it can be altered by isotopic fractionation during fixation (Kohl and Shearer, 1980; Steele et al., 1983). Therefore, it is necessary that the magnitude of isotopic fractionation during N_2 fixation be established before calculating the proportion of N_2 fixed, when the ^{15}N natural abundance technique is used (Steele et al., 1983; Shearer and Kohl, 1986; Ledgard, 1989).

Several studies have shown differences in ^{15}N natural abundance between plant parts (Shearer et al., 1980; Steele et al., 1983; Turner and Bergensen 1983; Bergensen et al., 1986; Ledgard, 1989). For example, Turner and Bergensen (1983) found ^{15}N enrichment of soybean plant parts in the following order: nodules > pods plus seeds > roots >whole plant > the foliage. This indicates that isotopic fractionation value for the part of the plant sampled should be determined for use in calculating the N_2 fixed. Other factors that influence the ^{15}N fractionation include the host plant and the rhizobial strain used (Bergensen et al., 1986; Yoneyama et al., 1986; Ledgard, 1989). Steele et al. (1983) examined some *Rhizobium* strains on more than one host plant

including soybean, siratro (*Macroptilium atropurpureum*) and lotus (*Lotus pedunculatus* L.) and found that the extent of isotopic fractionation was dependent on host plant and the infecting rhizobial strain. Ledgard (1989) inoculated white clover (*Trifolium repens*) and red clover (*T. pratense*) separately with a single rhizobial strain and a mixture of field isolates and reported similar results. For these reasons Shearer and Kohl (1986) and Ledgard (1989) pointed out that isotopic fractionation during N_2 fixation should be determined for each host-*Rhizobium* combination. Therefore, the objective of this study was to determine the magnitude of isotopic fractionation during N_2 fixation for desi and kabuli chickpeas inoculated with *Rhizobium ciceri* strain CP39 or a mixture of strains 27A2, 27A7 and 27A9. These rhizobial strains were used in field studies described in Chapter 3; thus, the isotopic fractionation value for each host-*Rhizobium* combination is required in calculating the proportion of N_2 fixed based on ^{15}N natural abundance method.

4.2 Materials and methods

4.2.1 Rooting medium and preparation of nutrient solution

The experiment was conducted using Leonard jars (Vincent, 1970), consisting of a bottle (330 ml) with the bottom half cut off and inverted into a 1 litre Mason jar. A cotton lamp wick was inserted through the neck of the inverted bottle and extended from the top of the inverted bottle to the bottom of the Mason jar. A foam plug in the neck of the inverted bottle held the wick in place. The bottle was filled with washed Turface (Aimcor Consumer Products LLC, Buffalo Grove, IL). Each Mason jar was filled with 600 ml N-free nutrient solution (Hoagland and Arnon, 1938) consisting of the following: 1000 ml deionized H_2O, 0.27 g KH_2PO_4, 0.35 g K_2SO_4, 1.0 g $CaSO_4 \cdot 2H_20$, 0.25 g $MgSO_4 \cdot 7H_2O$, 4.0 mg H_3BO_3, 0.99 mg $MnCl_2 \cdot 4H_2O$, 0.58 mg $ZnSO_4 \cdot 7H_2O$, 0.125 mg $CuSO_4 \cdot 5H_2O$, 5.4 mg $FeCl \cdot 6H_2O$, and 0.10 mg $Na_2MoO_4 \cdot 2H_2O$. Each assembled Leonard jar was wrapped in aluminum foil and autoclaved for 1 h. The jars were cooled for 24 h before the seeds were planted.

4.2.2 Seed sterilization and inoculation treatment

Seeds of Myles desi chickpea and Sanford kabuli chickpea were surface-sterilized by shaking with 70% alcohol for 3 min and then with 3% sodium hypochloride for 3 min. The seeds were rinsed six times with sterile water and dried in a sterile laminar airflow hood. Sterile seeds were inoculated with peat-based inoculant containing either CP39 (ICARDA, Aleppo, Syria; and kindly formulated by MicroBio RhizoGen, Saskatoon, SK) or a mixture of three strains 27A2, 27A7 and 27A9 (LiphaTec Inc., Milwaukee, WI) at the recommended rate (Table 3.2). One seed was sown per jar by carefully punching a hole through the aluminum foil and placing the seed into the Turface with sterilized forceps.

4.2.3 Plant growth conditions

The experimental design was a randomized complete block with six replications for each chickpea type. The plants were grown in a growth chamber (Model PGR 15, Controlled Environments Ltd, Winnipeg, MB) with a 16-h daylength and mean day and night temperatures of about 25 and 18°C, respectively. Relative humidity was maintained between 60 and 65%. The light source consisted of Cool White VHO and GRO-LUX VS VHO fluorescent lamps in a ratio of 3 to 1, supplying photosynthetically active radiation (PAR) of approximately 560 ±10 μmol m^{-2} s^{-1} at the top of the canopy. Nutrient solution was replaced every ten days.

4.2.4 Harvesting and plant tissue analysis

Plants were harvested at flowering or physiological maturity. The roots were washed free of Turface under running tap water and the nodules were carefully removed. The whole plant and the nodules were dried at 60°C and weighed. The shoots harvested at flowering were milled to a < 2-mm particle size in a Wiley mill (Arthur H. Thomas Company, Philadelphia, PA) and then passed through a cyclone mill (Tecator model Cyclotec 1093) equipped with a 0.4-mm sieve. Subsamples of ground materials were further finely ground in a rotating ball-bearing mill and approximately 1-mg samples were analyzed for total N and ^{15}N natural abundance as described in section 3.2.3. For the harvest at physiological maturity, the seeds were

ground for total N content and atom % ^{15}N excess. ^{15}N natural abundance was calculated as in section 3.2.4.

4.2.5 Statistical analysis

Data for the desi chickpea and the kabuli chickpea were analyzed separately, using the General Linear Model (SAS Institute, 1996). The least significant difference at 5% level was used for mean comparisons.

4.3 Results

The non-inoculated plants were not analyzed since few of these plants had nodules. The dry weight of nodules formed by the inoculant strains did not differ significantly in either the desi chickpea or the kabuli chickpea at the flowering stage (Table 4.1) or at physiological maturity (Table 4.2). Likewise, plant dry matter production did not differ between the inoculant strains at either sampling date. In the kabuli chickpea only, N accumulation was lower in the mixed-strain inoculant than the single strain inoculant (CP39), although the difference was significant only at the flowering stage.

The $\delta^{15}N$ values of the above-ground parts of both the desi chickpea and the kabuli chickpea at the flowering stage were not significantly different between the two inoculants (Table 4.1). However, the mixed inoculants resulted in a lower $\delta^{15}N$ values and a lower isotopic fractionation for the harvested seeds in the desi chickpea, but not in the kabuli chickpea (Table 4.2). In all cases, the $\delta^{15}N$ values for the shoots (Table 4.1) were lower than for the harvested seeds (Table 4.2). For example, inoculating the desi chickpea with the mixed-strains resulted in $\delta^{15}N$ values of –0.5475 for the harvested seeds compared to –1.3067 for the shoot harvested at flowering. Similarly, the corresponding $\delta^{15}N$ values for the harvested seeds and shoot when desi chickpea was inoculated with strain CP39 were –0.9062 and –1.9226, respectively.

The N from the seed from which the plants were grown represented about 5.8% of the total plant N at physiological maturity. The $\delta^{15}N$ of the seed N for the desi chickpea and the kabuli chickpea were 1.5078 and 2.1391, respectively. Hence, the $\delta^{15}N$ values for the harvested seeds and shoots were adjusted for the initial seed N,

using the following formula (Shearer and Kohl, 1993; S. F. Ledgard, personal communication):

$$\frac{\delta^{15}N\text{plant} \times N\text{plant} - \delta^{15}N\text{seed} \times N\text{seed}}{N\text{plant} - N\text{seed}} = \delta^{15}N_{adjusted} \qquad [4.1]$$

where $\delta^{15}N_{plant}$ is $\delta^{15}N$ of the plant part, $\delta^{15}N_{seed}$ is $\delta^{15}N$ of seed from which the plants were grown, N_{plant} and N_{seed} are the N yield of the plant and seed, respectively. Based on the adjusted $\delta^{15}N$ values, the isotopic fractionation coefficients (β) were estimated using the relationship suggested by Kohl and Shearer (1980).

$$\beta = 1 - \frac{1}{1000}(\delta^{15}N_{source} - \delta^{15}N_{adjusted}) \qquad [4.2]$$

The $\delta^{15}N_{source}$ is $\delta^{15}N$ of the atmospheric N_2, which is zero (Kohl and Shearer, 1980).

The isotopic fractionation coefficient (β) for the single strain CP39 was higher than for the mixed strains for the desi chickpea at physiological maturity (Table 4.2). The values were higher at the flowering stage (Table 4.1) than at physiological maturity (Table 4.2).

Table 4.1 Nodule dry weight, dry matter yield, N yield, ^{15}N abundance of above-ground parts and the isotopic fractionation factor for N_2 fixation in desi and kabuli chickpea inoculated with inoculants containing either strain CP39 or a mixture of strains 27A2, 27A7 and 27A9. Plants were grown in N-free medium solution and harvested at the flowering stage. The ^{15}N abundance values were adjusted for the $\delta^{15}N$ and amount of N in the seed from which the plants were grown.

Rhizobium strain	Nodule dry wt (mg plant^{-1})	Plant dry matter (g plant^{-1})	N yield (mg plant^{-1})	$\delta^{15}N$	β
	----------Desi----------				
27A2+27A7+ 27A9	153.62	1.83	48.83	-1.3067	1.0013
CP 39	168.60	2.21	48.41	-1.9226	1.0019
	----------Kabuli----------				
27A2+27A7+ 27A9	152.97	1.83	43.55*	-2.8225	1.0028
CP 39	234.40	3.31	81.97	-1.9496	1.0019

* Differences between 27A2+27A7+27A9 and CP39 were significant at the 0.05 level.

Table 4.2 Nodule dry weight, dry matter yield, N yield, ^{15}N abundance of harvested seeds and the isotopic fractionation factor for N_2 fixation in desi and kabuli chickpea inoculated with inoculants containing either strain CP39 or a mixture of strains 27A2, 27A7 and 27A9. Plants were grown in N-free medium solution and harvested at physiological maturity. The ^{15}N abundance values were adjusted for the $\delta^{15}N$ and amount of N in the seed from which the plants were grown.

Rhizobium strain	Nodule dry wt (mg plant^{-1})	Plant dry matter (g plant^{-1})	N yield (mg plant^{-1})	$\delta^{15}N$	β
	----------Desi----------				
27A2+27A7+					
27A9	225.3	4.47	137.99	-0.5475*	1.0005*
CP 39	254.0	4.59	136.77	-0.9062	1.0009
	----------Kabuli----------				
27A2+27A7+					
27A9	495.9	8.17	259.99	-0.8351	1.0008
CP 39	377.0	9.48	345.00	-0.6937	1.0007

* Differences between 27A2+27A7+27A9 and CP39 were significant at the 0.05 level.

4.4 Discussion

The data on nodulation and dry matter yield indicate that the inoculated plants grown hydroponically were comparable to field-grown chickpea inoculated with the same inoculant strains. The inoculant strain CP39 did not differ from the three-strain mixture in nodulation or dry matter yield, confirming the earlier observations in the field.

The $\delta^{15}N$ in the total N accumulated by the nodulated chickpea reflects isotopic fractionation during the N_2-fixing process, if adjustments are made for the initial N present in the seeds from which the plants were grown and for any extraneous N that may have been assimilated by the plant during culture (Bergensen et al., 1988;

Doughton et al., 1992). According to Shearer and Kohl (1986, 1993) the isotopic fractionation factor (β) during N_2 fixation is given by:

$$\beta_{N_2\text{-fixation}} = \frac{^{15}N/^{14}N \text{ atmospheric } N_2}{^{15}N/^{14}N \text{ fixed N}} \qquad [4.3]$$

It is small but important, when the ^{15}N natural abundance method is used to estimate the proportion of N derived from fixation. In the present study, values for the isotopic fractionation factor calculated from the $\delta^{15}N$ values for the above ground portions at flowering and for the harvested seed at physiological maturity are shown in Tables 4.1 and 4.2, respectively. None of the differences were significant at the flowering stage (Table 4.1). However, for the desi chickpea, inoculated with rhizobial strain CP39 and multi-strain inoculant (27A2, 27A7 and 27A9), the $\beta_{N_2\text{-fixation}}$ values after adjustment for the seed N were 1.0013 and 1.0019 at flowering, respectively, whereas the corresponding values for the kabuli chickpea at flowering were 1.0028 and 1.0019, respectively. If the $\delta^{15}N$ values, averaged over chickpea genotypes are used, the $\beta_{N_2\text{-fixation}}$ values are 1.0021 and 1.0019 for rhizobial strain CP39 and the multi-strain inoculant, respectively. These values are similar to those reported for shoots of the desi chickpea cultivars Tyson and Amethyst (Doughton et al., 1995 and Peoples and Turner [unpubl., according to Herridge et al., 1995]). The $\delta^{15}N$ value for Tyson desi chickpea was -2.10‰ (Doughton et al., 1995), whereas that for Amethyst desi chickpea was -1.65‰ (People and Turner, cited by Herridge et al., 1995), giving $\beta_{N_2\text{-fixation}}$ values of 1.0021 and 1.0017, respectively.

At physiological maturity, the harvested seeds were less depleted in ^{15}N abundance compared to the above-ground portion at flowering. Although this cannot be said of the above-ground parts at physiological maturity, various authors (e.g., Kohl and Shearer, 1980; Turner and Bergensen, 1983; Bergensen et al., 1988; Ledgard, 1989) have shown differences in ^{15}N abundance between plant parts. The $\beta_{N_2\text{-fixation}}$ values obtained for the harvested seed were 1.0005 and 1.0009 (significantly different) for the single strain and multi-strain inoculant, respectively in the desi chickpea (Table 4.2). For the kabuli chickpea, the respective values are 1.0008 and 1.0007. If the mean $\delta^{15}N$ values for the rhizobial strains are used, the $\beta_{N_2\text{-fixation}}$ values are 1.0007 and 1.0008 for strain CP39 and mixed strains (27A2, 27A7 and 27A9), respectively. No

report is available in the literature on isotopic fractionation for chickpea seeds harvested from hydroponically-grown plants, but these values are comparable to $\beta_{N_2\text{-fixation}}$ values of 1.0008, 1.0009 and 1.0010 reported by Bergensen et al. (1988) for entire plants of the soybean cultivars Lincoln, Forrest and Bragg.

Doughton et al. (1992) suggested that to account for seed N and extraneous N sources in isotopic fractionation estimation, non-inoculated plants should be grown in isolation from and under similar conditions as the inoculated plants. The total N and ^{15}N abundance of the nodulated plants minus the values from the non-nodulated plants provide adjustments for both the initial seed N and extraneous N. Although the initial seed N was accounted for in the present study, any extraneous N (from the putatively N-free culture medium), that might have been assimilated by the nodulated plants, was not accounted for because most of the non-inoculated plants did not grow beyond the expected stage from the nutrients provided by the seed. Thus, the isotopic fractionation factors in the present study assumed that the plants assimilated no or negligible amounts of extraneous N. In a similar study, Kohl and Shearer (1980) concluded that the contribution of extraneous N sources was essentially nil. Therefore, the β values reported in this study represent reliable isotopic fractionation factors for desi and kabuli chickpea nodulated by either rhizobial strain CP39 or a mixture of the strains 27A2, 27A7 and 27A9.

Several investigators, including Steele et al. (1983), Yoneyama et al. (1986) and Ledgard (1989), have reported that host plants and rhizobial strain can influence isotopic fractionation during N_2 fixation. For example, in the study by Ledgard (1989), using white clover and red clover inoculated with *R. leguminosarum* strain PDD 2668 or a mixture of rhizobia isolated from the field, the $\delta^{15}N$ of the shoots was larger for the rhizobial strain from the field than for strain PDD 2668. Thus, N_2 fixation would have been over-estimated, if the $\delta^{15}N$ value for PDD 2668 had been used in calculation from the field site. The $\delta^{15}N$ of the desi chickpea seed in the present study support this conclusion. The isotopic fractionation (β) value was higher for the single strain CP39 than for the mixed strains (27A2, 27A7 and 27A9) (Table 4.2). Thus, N derived from fixation for the seed would have been over-estimated, if the (β) value for the mixed strains had been used in calculations on plants inoculated with the single

strain. In contrast, the isotopic fractionation values for the kabuli chickpea and rhizobial strain combinations were similar, indicating that an accurate estimate of N_2 fixation would have been obtained from any of the values. Furthermore, the β value of either the desi shoot or the kabuli shoots at flowering for each inoculant did not differ and would produce essentially the same proportion of N_2 fixed, if any of the values is used in ^{15}N natural abundance calculation on chickpea shoots.

In soils with indigenous rhizobia, the strains of rhizobia that infect the host legume may vary (Ledgard, 1989; Doughton et al., 1992). Under those conditions, it is likely that the β value determined in the greenhouse or growth chamber may not be appropriate for estimating N_2 fixation in the field. The field used in N_2-fixation studies was free from indigenous rhizobia for chickpea (Rennie et al., 1982; Hynes et al., 1995) and, since the chickpea-*Rhizobium* symbiosis is very specific (Silsbury, 1989), it is likely the plants were infected entirely by the inoculant strains. Thus, the isotopic fractionation factors that were used for the calculation of the proportion of N_2 fixed, were appropriate.

Although grouping the chickpea cultivars in separate experiments prevented a direct comparison between the desi and the kabuli types, it was clear that the host genotype did not influence the isotopic fractionation factor. This is contrary to previous observations (Steele et al., 1983; Ledgard, 1989). Steele et al. (1983), using a number of host plants and host-*Rhizobium* combinations, found considerable variation in isotopic fractionation among plant species. For example, in *Lotus* and *Macroptilium* grown with rhizobial strain PDD 4683, the isotopic fractionation factors calculated for the foliage were 0.9995 and 1.0003, respectively. The results of the present study, therefore, suggest that the same β value would be appropriate for the calculation of the proportion of N_2 derived from the atmosphere by Myles desi and Sanford kabuli chickpeas inoculated with the same rhizobial strain.

5. TIME COURSE OF N_2 FIXATION AND GROWTH OF CHICKPEA

5.1 Introduction

Most estimates indicate that chickpea can derive between 26 and 83% of its N requirements from fixation (Evans et al., 1989; Beck et al., 1991; Herridge et al., 1995; Hossain et al., 1995). The large variation in the proportion or amount of N_2 fixed is due to many interacting factors, including environmental variables, host genotype, rhizobial strain, root nodule position and the length of time the plant actively supports N_2 fixation (Rennie and Kemp, 1984; George et al., 1987; Hardarson et al., 1989; Vessey, 1992).

Several studies have shown that N_2 fixation in nodulated grain legumes declines during seed development (e.g., Latimore et al., 1977; Deibert et al., 1979; Imsande, 1989). Lawn and Brun (1974) and Quebedeaux et al. (1975) reported a marked decline in symbiotic N_2-fixing activity at the onset of pod filling in soybean. On the other hand, Zapata et al. (1987) reported low initial N_2 fixation levels in field-grown soybean until the beginning of the reproductive stage (74 d after planting), but this high level of fixation was maintained for only 20 days. Similar observations in the decline of N_2 fixation during the early pod-filling stage have been reported for other legumes, including pea (Bethlenfalvay and Phillips, 1977; Dean and Clark, 1980; Vessey, 1992), common bean (Bethlenfalvay and Phillips, 1977, Pena-Cabriales et al., 1993) and bambara groundnut (Kumaga et al., 1994). This apparent decline has been linked to the carbohydrate deprivation hypothesis, which attributes the decrease in nodule function to a diminished supply of photosynthate to the nodules (Lawn and Brun, 1974; Latimore et al., 1977; Quebedeaux et al., 1975). However, work on irrigated soybean (Bergensen et al., 1989; 1992) has shown that this may not be case, because high rates of N_2 fixation continued throughout pod-fill. Contrary to the carbohydrate limitation hypothesis are also the recent findings by Stanforth et al.

(1994) which indicated that total N accumulation rate and accumulation per unit dry weight of nodule of field-grown plants remained constant or increased throughout the reproductive period in faba bean. In addition, considerable evidence indicates that N_2 fixation is maintained for longer periods into the reproductive stage in nodules located on the lower part of the root system compared to the crown region (McDermott and Graham, 1989; Wolyn et al, 1989; Vikman and Vessey, 1992, 1993). If lateral root nodules on chickpea roots maintain activity during the reproductive phase, it would be expected that a method of inoculation that induces lateral root nodulation would prolong the period of active N_2 fixation and, thus, enhance both the amount of N_2 fixed and the consequent seed yield. Therefore, the objective of the present study was to examine the time course of N_2 fixation and growth of desi chickpea under a controlled-environment by comparing seed-inoculated plants to plants grown in soil inoculated with granular inoculant.

5.2 Materials and methods

5.2.1 Growth medium

The study was conducted in growth chambers (Model PGV 36, Controlled Environments Ltd, Winnipeg, MB) in special pots constructed of 10-cm-diameter by 36-cm-long sections of polyvinyl chloride (PVC) pipe. Cheesecloth and a paper coffee filter held in place by a rubber band supported the bottom of each pipe. The pot was then placed in a 2-cm-deep plastic saucer. The pots were filled with a mixture of soil, industrial sand (Unimin Corporation, New Caanan, CT) and vermiculite (Vil Vermiculite, Toronto, ON) in a 2:1:1 ratio (v/v). The soil was collected in August 1998 from Outlook, SK, from one of the experimental sites used for the field studies (Chapter 3). After removing and discarding the top 3-cm layer, the soil was excavated to a depth of about 15 cm. The soil was dried and sieved, using a 6-mm screen, before mixing with the required proportion of sand and vermiculite. Each pot contained 4.5 kg of growth medium.

5.2.2 Seed sterilization and inoculation treatment

Seeds of desi chickpea cv. Myles were surface-sterilized by immersing in 70% alcohol for 3 min, followed by immersion in 3% sodium hypochloride solution for 3 min. The seeds were then rinsed six times with sterile water and, dried in a sterile laminar airflow hood. A sample of seeds was inoculated with either a liquid or peat-based inoculant. Inoculant preparations of *Rhizobium ciceri* strain CP39 (ICARDA, Aleppo, Syria; and kindly formulated by MicroBio RhizoGen, Saskatoon, SK) were applied at the recommended rates to deliver approximately 10^5 cells $seed^{-1}$. The four inoculation treatments were the non-inoculated control, seed-applied liquid inoculant, seed-applied peat inoculant or granular inoculant applied 2.5 cm below the seed. For the liquid formulation, the application rate was 4.5 ml kg^{-1} seed, whereas the peat-based seed-applied formulation was applied at 1.95 g kg^{-1} seed, using 5 ml of 1% gum arabic solution as adhesive. For the 2.5 cm below seed-placement treatment, soil to the desired depth was removed, and the granular inoculant (60 mg pot^{-1}) was spread on the soil surface and the soil was then replaced.

5.2.3 Growth conditions

Four seeds were planted per pot at a depth of 3 cm, and the pots were placed in the growth chamber. Growth chamber conditions were maintained at a 16-h daylength and a mean day and night temperatures of 25 and 18°C, respectively. Relative humidity was maintained between 60 and 70%. The light source was composed of Cool White VHO and GRO-LUX VS VHO fluorescent lamps at a ratio of 3 to 1, supplying photosynthetic active radiation (PAR) of approximately 560 ±10 μmol m^{-2} s^{-1} at the top of the canopy. The pots were arranged in a randomized complete block design with four replications. After emergence, the seedlings in each pot were thinned to two after which a 25 ml solution, containing 10 mg of 10.5% ^{15}N enriched $^{15}NH_4{}^{15}NO_3$, was applied to the surface of the soil of each pot. Flax was also grown in separate pots for estimation of N_2 fixation by the ^{15}N-enrichment technique. The plants were maintained at field capacity by daily addition of tap water, and weekly addition of 100 ml half-strength N-free Hoagland nutrient solution (Hoagland and Arnon, 1938) per pot. To minimize the passive downward washing of rhizobial cells by

percolating water or nutrient solution from above. watering was by capillary rise of water or nutrient solution from the plastic saucer under each pot.

The experiment was repeated with similar inoculation treatments and grown under similar conditions except that the 25 ml solution containing 10 mg 10.5% ^{15}N enriched $^{15}NH_4{}^{15}NO_3$, was applied by capillary rise from the plastic saucer under each pot at planting.

5.2.4 Harvesting and plant tissue analysis

The plants were harvested at 28, 42, 56, 70, 84 or 98 days after planting (DAP), corresponding to the late vegetative stage, flowering, early pod-filling, mid pod-filling, late pod-filling and physiological maturity, respectively. The roots were carefully washed under running tap water, and the crown and lateral root nodules were removed separately. Nodules were counted and dried with the whole plant at 60°C for 7 d. The dry weight of nodules and the plant dry matter yield were determined. The above-ground plant parts (leaves + stems, and pods in later harvests) of chickpea and flax were milled to a < 2-mm particle size in a Willey mill (Arthur H. Thomas Company, Philadelphia, PA) and then passed through a cyclone mill (Tecator model Cyclotec 1093) equipped with a 0.4-mm sieve. Subsamples of ground materials were further finely ground in a rotating ball-bearing mill and approximately 1-mg samples were analyzed for percentage N and atom percent ^{15}N excess, using continuous flow isotope ratio mass spectrometer (Europa Scientific, Crewe, England) interfaced with Roboprep sample converter (Europa Scientific). The working standard was ^{15}N-enriched pea residue with an atom %^{15}N content of 0.6013 and standard deviation of 0.0007. For the final harvest, i.e., at physiological maturity, seeds of both chickpea and flax were also analyzed. Chickpea seed protein was determined by converting the total N to % protein using the factor 6.25 (Tkachuk, 1969) and then expressed as protein concentration. The percentage of plant N derived from the atmosphere (%Ndfa) was estimated using the ^{15}N isotope dilution method and was calculated according to Rennie and Dubetz (1986) as follows:

$$\%\,\text{Ndfa} = \left(1 - \frac{\text{atom}\,\%\,{}^{15}\text{N excess N}_2\text{-fixing crop}}{\text{atom}\,\%\,{}^{15}\text{N excess non-fixing crop}}\right)100 \qquad [5.1]$$

5.2.5 Statistical analysis

Data for each sampling date were analyzed separately for each experiment in addition to the combined analyses over experiments for each sampling date, using the General Linear Model (SAS Institute, 1996). In the analyses, inoculation treatment was considered a fixed factor, whereas the experiments were considered random variables with replications nested within experiments. Planned comparisons among treatments were made, using contrasts. For some of the parameters measured, e.g., seed protein concentration and percent N derived from the atmosphere (%Ndfa) for the seed, the overall F tests for treatments were not significant. However, partitioning of the treatment degrees of freedom into single degree of freedom contrasts indicated that some of the treatments differed significantly. According to Chew (1977), it is not necessary to carry out an F test when comparisons among treatments means are planned; a view supported by Steel et al. (1997). In comparing treatments, the overall F test is averaged over the possible comparisons. Thus, if only one or two of these contrasts are significant, the overall F test is diluted or weakened by the non-significant contrasts and erroneously may give a non-significant F value.

5.3 Results

5.3.1 Nodulation

The inoculation treatments produced similar results with no significant differences between the two experiments for total number of nodules (Table 5.1) or nodule dry weight at any of the sampling dates (Table and 5.2). The interactions between experiment and inoculation treatment for nodule numbers and nodule dry weight at all sampling dates were not significant, except for nodule numbers and dry weight at physiological maturity (98 DAP) and early pod-filling (56 DAP), respectively. Hence, the data were averaged over experiments (Table 5.3). Inoculation method significantly influenced the position of nodule formation on the roots in both experiments. The peat-based inoculants (applied to the seed) produced nodules primarily at the crown region, whereas most of the nodules formed by the granular inoculant were located on the lateral roots. The liquid-formulated inoculant (applied to the seed) formed about the same number of nodules on the lateral roots as on the

crown region. Averaged over the two experiments, total nodule numbers were not significantly different between the peat and granular inoculants, but both were, in general, significantly higher than that for the liquid and the non-inoculated control. Total nodule numbers for the peat inoculant treatment increased from 2 plant^{-1} at the late vegetative stage (28 DAP) to 4.75 plant^{-1} 56 DAP and then declined toward physiological maturity of the plant (Table 5.3). On the other hand, the total number of nodules formed by the granular inoculant increased over three-fold from 1.07 plant^{-1} at 28 DAP to 3.63 plant^{-1} during a 4-week period, and maintained a similar number of nodules to physiological maturity of the plant.

Nodulation was generally poor as compared to that observed in field-grown Myles desi chickpea (Section 3.3.1.1, Chapter 3), particularly in Experiment 1. In this experiment, no nodulation was observed in the liquid inoculant treatments until the mid pod-filling stage (70 DAP), when some nodules were found on the lateral roots (Appendix 30). In all the inoculant treatments and at all sampling dates, total nodule numbers were higher in Experiment 2 (Appendix 31) than Experiment 1. Unlike Experiment 1, the total number of nodules formed by the granular inoculant in Experiment 2 after the late vegetative stage remained fairly constant until physiological maturity.

Tables 5.1. Mean squares from the analysis of variance for nodule numbers of desi chickpea from various inoculation treatments at different growth stages in two experiments.

Sources of variation	d.f	Days after planting (DAP)†					
		28	42	56	70	84	98
Exp	1	15.82	10.05	4.13	11.28	0.63	9.57
Reps in exp	6	0.67	0.62	3.88	3.91	3.49	0.51
Inoculation	3	5.88	25.43*	28.72	30.09**	21.53*	26.67*
Non-inoc. vs. inoc.	1	8.46	29.26*	11.00	243.80*	34.44**	30.94*
Peat vs. Liquid	1	9.00	40.64*	70.14	111.47	21.39*	25.00*
Granular vs. peat	1	3.50	1.00	5.06	62.53	0.63	3.06
Granular vs. liquid	1	1.26	28.89*	37.52	340.98*	23.77*	45.56*
Exp x inoc.	3	4.40	2.56	14.26	0.93	1.03	2.63**
Error	18	0.20	1.12	6.21	2.75	1.86	0.54
Total	31						

*,** Significant at the 0.05 and 0.01 levels, respectively.
† 28 DAP = late vegetative stage, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Table 5.2. Mean squares from the analysis of variance for nodule dry weight of desi chickpea from various inoculation treatments at different growth stages in two experiments.

Source of variation	d.f	Days after planting (DAP)†					
		28	42	56	70	84	98
Exp	1	0.005	0.013	0.003	0.014	0.001	0.007
Reps in exp	6	0.000	0.002	0.005	0.005	0.014	0.009
Inoculation	3	0.001	0.029	0.003	0.029**	0.050	0.047*
Non-inoc. vs. inoc.	1	0.002	0.026	0.025	0.041**	0.104*	0.042*
Peat vs. Liquid	1	0.001	0.022	0.040	0.040**	0.015	0.044*
Granular vs. peat	1	0.000	0.000	0.001	0.001	0.009	0.009
Granular vs. liquid	1	0.001	0.024	0.055	0.030**	0.047	0.095**
Exp x inoc.	3	0.001	0.003	0.007*	0.001	0.006	0.002
Error	18	0.000	0.001	0.002	0.003	0.015	0.004
Total	31						

*,** Significant at the 0.05 and 0.01 levels, respectively.
† 28 DAP = late vegetative stage, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Table 5.3. Nodule numbers of desi chickpea from various inoculation treatments at different growth stages, averaged over two experiments.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	Number of nodules plant^{-1}					
	Crown nodules					
Peat	1.44	2.44	3.50	3.75	2.57	2.69
Liquid	0.25	0.07	0.19	0.19	0.25	0
Granular	0	0	0	0	0	0
Non-inoculation	0	0	0	0	0	0
$LSD_{(0.05)}$	ns	1.51	1.87	1.33	0.74	2.39
	Lateral root nodules					
Peat	0.56	1.06	1.25	0.63	0.75	0.25
Liquid	0.25	0.25	0.38	0.44	0.75	0.44
Granular	1.07	3.00	3.63	3.19	3.44	3.82
Non-inoculation	0	0.07	1.63	0.44	0.19	0.13
$LSD_{(0.05)}$	ns	1.61	ns	2.09	1.91	1.79
	Total					
Peat	2.00	3.50	4.75	4.38	3.32	2.94
Liquid	0.50	0.32	0.57	0.63	1.00	0.44
Granular	1.07	3.00	3.63	3.19	3.44	3.82
Non-inoculation	0	0.07	1.63	0.44	0.19	0.13
$LSD_{(0.05)}$	ns	2.55	ns	1.53	1.61	2.58
Contrasts‡						
Non-inoc vs. inoc	1.19	2.20*	1.35	2.29**	2.40**	2.27*
Peat vs. liquid	1.50	3.18*	4.18	3.75**	2.32*	2.50*
Granular vs. peat	0.93	0.50	1.12	1.19	0.12	0.88
Granular vs. liquid	0.57	2.68*	1.06	2.56**	2.44*	3.38*

*,** Significant at the 0.05 and 0.01 levels, respectively.

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

‡ Differences between specified treatments.

Nodule dry weight data were similar to nodule number data. Peat-based inoculant (applied to the seed) resulted in most of the nodule dry weight in the crown, granular inoculant (applied to the soil) resulted in most of the nodule dry weight on the lateral roots and nodule dry weight from the liquid inoculant (applied to the seed) did not differ from the non-inoculated treatment (Table 5.4). The total nodule dry weight increased to a peak at late pod-filling (84 DAP) for all inoculant treatments and then decreased toward the physiological maturity of the plant. The greatest increase in total nodule dry weight for the peat inoculant occurred between the late vegetative and flowering stages (28 – 42 DAP), whereas that for the liquid and granular inoculants occurred between the mid pod-filling and late pod-filling stages (70 – 84 DAP). During these periods, total nodule dry weight for the peat, liquid and granular inoculants increased by 68.5, 56.4 and 77.4 mg plant^{-1}, respectively. Total nodule dry weights were similar for the peat and granular inoculants until the mid pod-filling stage (70 DAP) after which the granular inoculant treatment accumulated much more nodule dry matter than the peat inoculant. Seed inoculation with liquid-formulated inoculant produced low total nodule dry weight that was not significantly different from the non-inoculated control at all sampling dates. Unlike the granular inoculant, total nodule dry weight for the peat and liquid inoculants were generally lower in Experiment 1 (Appendix 32) than in Experiment 2 (Appendix 33). For granular inoculation, nodule dry matter in Experiment 2 was higher than in Experiment 1 at the initial growth stages, but the reverse was true from mid pod-filling onward.

Table 5.4. Nodule dry weight of desi chickpea from various inoculation treatments at different growth stages, averaged over two experiments.

Inoculation treatment	Days after planting (DAP)[†]					
	28	42	56	70	84	98
	Crown nodules (mg plant^{-1})					
Peat	21.4	58.3	87.1	117.2	86.6	104.1
Liquid	7.9	2.3	14.7	15.2	4.0	0
Granular	0	0	0	0	0	0
Non-inoculation	0	0	0	0	0	0
$LSD_{(0.05)}$	ns	36.9	55.0	70.7	17.9	40.4
	Lateral root nodules (mg plant^{-1})					
Peat	4.1	32.5	47.1	18.6	67.6	29.7
Liquid	1.7	14.2	19.4	21.0	88.6	28.8
Granular	24.8	93.3	151.3	123.4	200.8	183.2
Non-inoculation	0	1.5	42.6	15.8	17.8	31.6
$LSD_{(0.05)}$	ns	82.4	83.4	32.1	130.9	55.6
	Total nodule dry wt (mg plant^{-1})					
Peat	25.5	90.8	134.2	135.7	154.1	133.7
Liquid	9.6	16.4	34.1	36.1	92.5	28.8
Granular	24.8	93.3	151.3	123.4	200.8	183.2
Non-inoculation	0	1.5	42.6	15.8	17.8	31.6
$LSD_{(0.05)}$	ns	87.8	ns	36.3	126.9	75.8
Contrasts[‡]						
Non-inoc vs. inoc	20.0	65.3	63.9	82.6**	131.3*	83.6*
Peat vs. liquid	15.9	74.4	100.1	99.6**	61.6	104.9*
Granular vs. peat	0.7	2.5	17.1	12.3	46.7	49.5
Granular vs. liquid	15.2	76.9	117.2	87.3**	108.3	154.4**

*,** Significant at the 0.05 and 0.01 levels, respectively.

[†] 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

[‡] Differences between specified treatments.

5.3.2 Dry matter accumulation in desi chickpea

Dry matter production of desi chickpea in Experiment 1 did not differ from that in Experiment 2 until the late pod-filling stage (84 DAP) (Table 5.5), when it was higher in Experiment 1 than Experiment 2 (Appendices 34 and 35). In general, dry matter yield of the chickpea plant increased throughout the growth cycle in all the treatments (Table 5.6). Averaged over experiments, the increase in dry matter accumulation was greatest between the late vegetative (28 DAP) and the early pod-filling (56 DAP) stages. Dry matter accumulation after the early pod-filling stage (56 DAP to 98 DAP) was higher for the granular inoculant than for the other inoculant treatments. Significant differences among inoculation treatments for dry matter yield were observed at the late vegetative (28 DAP) and late pod-filling stages (84 DAP) (Tables 5.5 and 5.6). The experiment by inoculation treatment interaction was not significant, except at physiological maturity (Table 5.5), due primarily to the high yield for the non-inoculated control in Experiment 1 (Appendix 34), relative to the Experiment 2 (Appendix 35).

Table 5.5. Mean squares from the analysis of variance for dry matter production of desi chickpea from various inoculation treatments at different growth stages in two experiments.

Source of variation†	d.f	Days after planting (DAP)‡					
		28	42	56	70	84	98
Exp	1	0.447	0.843	4.914	14.824	26.110**	33.140**
Reps in Exp	6	0.024	0.209	0.435	0.435	0.356	0.514
Inoculation	3	0.035	0.101	0.861	0.985	3.387**	4.368
Non-inoc vs. inoc	1	0.012	0.025	0.838	2.036*	5.541**	2.838
Peat vs. liquid	1	0.052	0.143	0.929	0.874	1.815*	3.303
Gran vs. peat	1	0.003	0.017	0.090	0.081	0.603	1.894
Gran vs. liquid	1	0.082*	0.259	1.597	0.424	4.510**	10.200
Exp x inoc.	3	0.007	0.034	0.172	0.209	0.097	1.285*
Error	18	0.023	0.113	0.309	0.429	0.167	0.314
Total	31						

*,** Significant at the 0.05 and 0.01 levels, respectively.

† Non-inoc = non-inoculated, inoc = inoculated. Gran = granular

‡ 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Table 5.6. Dry matter production of desi chickpea from various inoculation treatments at different growth stages, averaged over two experiments.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	g plant^{-1}					
Peat	1.11	2.06	3.02	3.69	3.77	4.12
Liquid	1.00	1.87	2.54	3.22	3.09	3.21
Granular	1.14	2.13	3.17	3.55	4.16	4.81
Non-inoculation	1.04	1.96	2.54	2.91	2.71	3.36
$LSD_{(0.05)}$	0.13	ns	ns	0.73	0.50	ns
Contrasts‡						
Non-inoc vs. inoc	0.04	0.06	0.37	0.58*	0.96**	0.69
Peat vs. liquid	0.11	0.19	0.48	0.47	0.68*	0.91
Granular vs. peat	0.03	0.07	0.15	0.14	0.39	0.69
Granular vs. liquid	0.14*	0.26	0.63	0.33	1.07**	1.60

*,** Significant at the 0.05 and 0.01 levels, respectively.
† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.
‡ Differences between specified treatments.

5.3.3 N_2 fixation

The proportions and the amounts of N_2 fixed did not differ significantly between the two experiments (Tables 5.7 and 5.8), except for the %Ndfa at the initial sampling date (28 DAP) (Table 5.7) which was higher in Experiment 1 than Experiment 2 (data not shown). The experiment by treatment interactions for %Ndfa and amount of N_2 fixed were also not significant at any of the sampling dates. Therefore, data for the combined analyses are presented in Tables 5.9 and 5.10.

Table 5.7. Mean squares from the analysis of variance for percentage N derived from the atmosphere throughout the growth cycle by desi chickpea from various inoculation treatments in two experiments.

Source of variation	d.f	Days after planting (DAP)†					
		28	42	56	70	84	98
Exp	1	2105**	51	881	3392	43	291
Reps in exp	6	75	239**	293*	664**	436*	197
Inoculation	3	284*	417	764	783*	738	838**
Non-inoc vs. inoc	1	460*	772	1300	975*	1364*	1895**
Peat vs. liquid	1	262	4	428	446	384	570*
Granular vs. peat	1	3	317	105	250	79	35
Granular vs. liquid	1	322*	396	955	1364*	813	321
Exp x inoc.	3	31	90	174	74	138	34
Error	18	42	42	101	86	121	116
Total	31						

*,** Significant at the 0.05 and 0.01 levels, respectively.
† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Table 5.8. Mean squares from the analysis of variance for amount of N derived from the atmosphere throughout the growth cycle by desi chickpea from various inoculation treatments in two experiments.

Source of variation	d.f	Days after planting (DAP)†					
		28	42	56	70	84	98
Exp	1	59.9	2.0	39.2	198	863	1413
Reps in exp	6	3.0	35.2*	88.2	179**	143	64
Inoculation	3	17.9	73.1	556.6*	641*	710**	556
Non-inoc vs. inoc	1	27.0	109.9*	660.6*	942*	910**	790
Peat vs. liquid	1	24.9	17.3	565.5*	383	545*	491
Granular vs. peat	1	1.9	38.8	40.3	130	118	35
Granular vs. liquid	1	13.1	108.1*	907.9*	958*	1168**	789
Exp x inoc.	3	5.3	10.6	42.2	68	30	112
Error	18	4.1	9.8	37.5	47	114	76
Total	31						

*,** Significant at the 0.05 and 0.01 levels. respectively.
† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Table 5.9. Percentage N derived from the atmosphere throughout the growth cycle by desi chickpea from various inoculation treatments averaged over two experiments.

Inoculation Treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
Peat	20.6	17.0	31.6	32.7	30.5	36.2
Liquid	12.5	16.0	21.2	22.2	20.6	24.2
Granular	21.5	25.9	36.7	40.6	34.9	33.2
Non-inoculation	9.4	8.3	15.1	19.1	13.6	13.4
$LSD_{(0.05)}$	8.9	15.1	21.0	13.7	18.7	9.3
Contrasts‡						
Non-inoc vs. inoc	8.8*	11.3	14.7	12.7*	15.1*	17.8**
Peat vs. liquid	8.1	1.0	10.4	10.5	9.9	12.0*
Gran vs. peat	0.9	8.9	5.1	7.9	4.4	3.0
Gran vs. liquid	9.0*	9.9	15.5	18.4*	14.3	9.0

*,** Significant at the 0.05 and 0.01 levels, respectively.

† 28 DAP = late vegetative stage, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

‡ Differences between specified treatments

Table 5.10. Amount N derived from the atmosphere throughout the growth cycle by desi chickpea from various inoculation treatments averaged over two experiments.

Inoculation Treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	mg plant^{-1}					
Peat	4.8	6.4	18.5	19.4	20.6	20.3
Liquid	2.3	4.2	6.6	9.6	8.5	9.3
Granular	4.1	9.5	21.7	25.1	25.6	23.3
Non-inoculation	1.6	2.5	5.1	5.5	5.8	6.2
$LSD_{(0.05)}$	ns	5.2	10.3	13.1	8.7	16.8
Contrasts‡						
Non-inoc vs. inoc	2.1	4.2*	15.5*	12.5*	12.4**	11.4
Peat vs. liquid	3.2	2.2	11.9*	9.8	12.1*	11.0
Gran vs. peat	0.7	3.1	3.2	5.7	5.0	3.3
Gran vs. liquid	2.5	5.3	15.1*	15.5*	17.1**	14.0

*,** Significant at the 0.05 and 0.01 levels, respectively.

† 28 DAP = late vegetative stage, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

‡ Differences between specified treatments

Among the inoculated treatments, %Ndfa differed significantly at 28, 70 and 98 DAP (Tables 5.7 and 5.9), whereas differences in the amount of N_2 fixed were significant at 56, 70 and 84 DAP (Tables 5.8 and 5.10), due to the low values for the liquid inoculant treatment (Tables 5.9 and 5.10). The proportions and amounts of N_2 derived from fixation were not different between the peat and granular inoculant treatments at all sampling dates. Averaged over experiments, the patterns of %Ndfa and N_2 fixed throughout the growth cycle of the inoculated and non-inoculated control treatments (Table 5.8) were similar to that of dry matter yield (Table 5.6). Generally, %Ndfa increased progressively from the late vegetative stage (28 DAP) to the mid pod-filling stage (70 DAP) and declined during the next growth stage (late pod-

filling). At the mid pod-filling stage, the %Ndfa for the granular inoculation was about double that for the liquid inoculant and the non-inoculation treatments.

In general, little N_2 was fixed by the late vegetative stage (28 DAP) in all treatments but increased four and almost six times for the seed-applied and soil inoculation treatments, respectively, by physiological maturity. The highest daily N_2 fixation rate (0.9 mg plant^{-1}) in the peat and granular inoculant treatments occurred between the flowering and early pod-filling stages (42-56 DAP), with little or no fixation after the early pod-filling stage. For the liquid inoculant, the highest daily N_2 fixation rate (0.23 mg plant^{-1}) occurred between the early and mid pod-filling stages (56-70 DAP).

5.4 Discussion

Chickpea is often reported to have a low capacity for N_2 fixation among the legume crops grown in a rotation-based cropping system (Papastylianou, 1987; Smith et al., 1987; Keatinge et al., 1988). The %Ndfa can range from 0 to 83%, depending on the method of assessment, host genotype, rhizobial strain, method of inoculation and environmental variables (Rennie and Dubetz, 1986; Papastylianou, 1987; Smith et al., 1987; Keatinge et al., 1988; Beck et al., 1991; Beck, 1992; Herridge et al., 1995; Hossain et al., 1995). In the present study, nodulation was delayed and generally was poor, which was reflected in low N_2 fixation in all the inoculation treatments.

Four weeks after seeding (late vegetative stage), the peat, liquid and granular inoculant had resulted in the formation of only 2.0, 0.5 and 1.0 nodules, respectively (Table 5.3), compared to an average of 13 and 27 reported by Silsbury (1989) and Minchin et al. (1980), respectively, for desi chickpea of the same age. Several factors may have contributed to the low levels of nodulation. For example, high temperature can cause the rapid death of rhizobia limiting nodule formation (Day et al., 1978; Graham and Rosas, 1978). Minchin et al. (1980) reported a drastic decline in nodule numbers in three chickpea genotypes when grown at 30/18°C compared with 22/18°C. Using cv. Chaffa desi chickpea inoculated with *Rhizobium* strain CC 1192,

Rawsthorne et al. (1985) also found that high temperature (32.5°C day/ 18°C night) delayed nodulation and nodule activity.

In the present study, the plants were grown at 25/18°C, not too high to affect nodulation adversely. Thus, the reason for the low nodulation could not be accounted for. However, the extremely poor nodulation observed in the liquid inoculant treatment indicated that environmental factors might have played a major role. Growing the chickpea in the growth room also may have resulted in sub-optimum conditions for growth and N_2 fixation. Hansen (1994) argued that the rhizobial strain is much more exposed to unfavourable environmental factors with liquid-formulated inoculum than peat-formulated inoculum. The data from field experiments, using the same chickpea cultivar and inoculants, also support this observation (Chapter 3).

Total nodule numbers were generally higher in the peat treatment than the granular inoculant treatment until the late pod-filling stage. At this stage, nodulation of the former declined, whereas that for the latter remained virtually the same or increased. The decline in total nodule numbers after the early pod-filling stage in the peat inoculant treatment was also evident in the liquid-inoculant treatment, indicating that, although nodule formation ceased during the later part of the growing cycle when the inoculant was seed-applied, nodulation continued in the soil-inoculated treatment. Thus, nodule formation in the soil-inoculation treatment was delayed relative to the seed-inoculation treatment, but this delay was compensated for by larger nodule dry weight. Smith et al. (1981) and Danso and Bowen (1989) also found that, when few nodules are produced on soybean roots, the nodules often grow much larger than when many nodules are formed. It is not surprising that root infection by the inoculant strain added to the soil was delayed because the inoculum was deposited 2.5 cm below the seed, and a time lag occurred before the developing legume root contacted the rhizobia. Unlike the seed inoculated plants, the lateral roots of plants grown in the soil inoculated treatment nodulated later in the growing season due to the availability of inoculant rhizobia at that soil depth. The 2.5-cm inoculant placement depth was chosen, based on the results of previous field study (Chapter 3). The study indicated that granular inoculant placed either 2.5 cm or 8 cm below the seed was superior to placement with the seed.

Nodule dry weight increased gradually from the late vegetative stage (28 DAP) to a maximum at the late pod-filling stage (84 DAP) and thereafter decreased during the final growth stage (Table 5.4). The decline in nodule dry weight during the later part of the growth cycle may be attributed to nodule senescence and a decrease in the availability of photosynthates for nodule metabolism. Such decreases may be due to the increased demand of developing fruits for assimilates (Rawsthorne et al., 1985). In common bean cv. Flor de Mayo, nodule dry weight decreased after 69 DAP (28 d before physiological maturity), whereas in cv. Bayocel, nodule dry weight was maintained until the final harvest (97 DAP) (Pena-Cabriales et al., 1993). Kumaga et al. (1994) also found that nodule dry weight of two bambara groundnut (*Vigna subterranea*) cultivars declined after the mid pod-filling stage (120 DAP). In the present study, the highest nodule dry matter accumulation for the peat-based inoculant occurred between the late vegetative and the flowering stages (28-42 DAP), whereas that for the liquid and granular inoculants occurred between the mid pod-filling and late pod-filling stages (70-84 DAP). These results are in contrast to nodulation of soybean (Danso et al., 1990) and bambara groundnut (Kumaga et al., 1994). In these studies the period of pronounced nodule growth in soybean was between the flowering and early pod-filling stages, whereas nodule growth in bambara groundnut was greatest between the late vegetative and early pod-filling stages. This illustrates the differences among legumes, among cultivars within the same crop or possibly among inoculant placements.

Differences in the growing conditions could also play a part in the observed differences in the nodule growth pattern. Total nodule dry matter for the peat inoculant was essentially the same as that for the granular inoculant by the mid pod-filling stage. Thereafter, nodule dry weight for the granular inoculant although not significant, was over 30% higher than that for the peat, emphasizing the fact that nodules produced by the former were younger and either had a higher dry matter accumulation rate or a lower rate of senescence than the latter. This contention is reflected in the nodulation pattern observed for the seed-applied inoculation and that for soil inoculation.

An aspect of the study was to examine the influence of inoculation method on the distribution of the nodules on the root system. On this basis, pots for growing the

chickpea plants were constructed from PVC pipes to obtain greater soil depth. This was to avoid a possible upward growth of the roots after they have reached the bottom of the pot, which could confound the position of the nodules in relation to the method of inoculation. In support of the field studies (Chapter 3) and that of others (Hardarson et al., 1989; Wadisirisuk et al., 1989; Danso and Bowen, 1989; Danso et al., 1990), inoculating the seed, particularly with peat inoculant, produced nodules predominantly at the crown region of the root, whereas inoculating the soil at 2.5 cm below the seed resulted in the formation of all the nodules at the lower part of the root system. On a nodule dry weight basis, the peat inoculant formed, on the average, between 64 and 86% of their nodules at the crown region throughout the growth cycle (Table 5.4). This compares well with Hardarson et al. (1989) who reported that inoculating soybean seed caused the formation of 87% of the nodules on the upper 5-cm section of the tap root, whereas inoculating the soil at the seeding level or 5 cm below the seed produced only 20-40% of the nodules at this root section. The position of the nodules observed in this study indicates that nodule formation is restricted to the vicinity of inoculant placement as suggested by Danso and Bowen (1989).

Nodulation results from the exposure of the rhizobial strain to root hairs of the host. Therefore, either the rhizobia must move to contact the root or the root must grow toward the rhizobia. It is well documented that *Rhizobium* do not move through the soil over large distances (Madsen and Alexander, 1982; Chamblee and Warren, 1990; Worrall and Roughley, 1991); thus the emerging root hairs of the host plant must contact the rhizobia (Date, 1991; Brockwell et al., 1995). Studies on the mobility of rhizobia in the rhizosphere have shown that percolating water plays a major role in the dispersal of rhizobial inoculum (Hamdi, 1971; Breitenbeck et al., 1988; Worrall and Roughley, 1991). In the present study, watering the plants from the bottom of the pot minimized passive movement of inoculant strain with flowing water. It can, therefore, be argued that inoculating the soil may increase the spatial distribution of the inoculant strain and possibly improve the chances of the developing root hairs contacting the inoculum. Failure of the inoculant to migrate away from the inoculated site was demonstrated in a dry soil by Brockwell and Whalley (1970). In this study, the authors observed that seed germination and root growth occurred without nodule

development, although large numbers of the inoculant rhizobia applied to the seed were recovered from the inoculated site.

The position of the nodules on the root system, rather than the number or fresh weight of nodules, influenced the amount of N_2 fixed by soybean plants (Hardarson et al., 1989). This observation has been associated with the age of the nodules, suggesting that nodules on the lower part of the root system or on lateral roots (which are often formed later than those at crown region) contribute significantly to N_2 fixation during the reproductive or later part of the growth cycle. In the present study, the differences in the proportions and amounts of N_2 fixed between the soil inoculation treatment with all the nodules located on the lateral roots and the seed inoculation with most of the nodules at the crown region were not significant at all sampling dates. Similarly, the dry matter yield was not different between the peat and the granular inoculant treatments at all sampling dates. These observations are consistent with the conclusion of Brockwell et al. (1988) that neither seed inoculation with peat-based inoculant nor soil inoculation with liquid inoculant is better than the other when environmental conditions are not limiting. The low N_2 fixed in the liquid inoculant treatment is most likely due to poor nodulation. The low N_2 fixation translated into lower dry matter yield at some growth stages.

The N demands of grain legumes are greatest during seed development (Lawn and Brun, 1974; Zapata et al. 1987; Imsande, 1989). However, several studies have shown that N_2 fixation declines with the onset of pod-filling (Lawn and Brun, 1974. Westermann et al., 1981; Wolyn et al., 1989; Vessey 1992). Using the acetylene reduction technique, Minchin et al. (1980) reported that N_2 fixation in chickpea reached a maximum around 45 DAP after which it declined to relatively low levels between 67 and 81 DAP. Similarly, Dart and Krantz (1977) observed that chickpea nodules showed a reduced nitrogenase activity soon after flowering. Evans (1982), using five chickpea genotypes grown in a controlled environment, found that maximum nitrogenase activity occurred during flowering and prior to, or during, initial seed formation. The data for the present study showed that N_2 fixing activity in cv. Myles desi chickpea increased from the late vegetative stage (28 DAP), generally

reaching a maximum at the early pod-filling stage (56 DAP) in both the inoculated and non-inoculated control plants, and then declined thereafter (Table 5.10).

The inconsistencies between the present study and others regarding the period of maximum N_2 fixation could be due to the methodological differences in the measurement (Attewell and Bliss, 1985), genotypic differences (Evans, 1982; Vessey, 1992) and the environmental conditions under which the plants were grown (Vessey, 1992). In all the studies mentioned above, N_2 fixation was assessed by the acetylene reduction technique. During sampling for acetylene reduction assay, some of the nodules on the lateral roots which contribute significantly to N_2 fixation during the reproductive phase (Wolyn et al., 1989; McDermott and Graham, 1989, Hardarson, 1993) could be lost. In the present study, however, the ^{15}N isotope enrichment method was used and, thus, concerns regarding loss of nodules during sampling do not apply.

Although chickpea genotypes CP156288, CP171180 and CP156296-b were similar in flowering, the peak nitrogenase activity for the former extended for a longer period than the other two (Evans, 1982). This was attributed to prolonged vegetative growth of CP156288 relative to the other two cultivars. A possible implication is that it would be advantageous to select cultivars having a longer vegetative phase in areas with a longer growing season, thus, prolonging the period of maximum nitrogenase activity (Rennie and Kemp, 1984).

The decline in N_2 fixation in soybean during the reproductive phase has been associated with the development of the pods as a competing sink, thereby limiting carbohydrate availability to the root nodules (Lawn and Brun, 1974; Latimore et al., 1977). As in soybean, chickpea pods develop a strong sink for assimilates and this may decrease the available carbohydrate necessary to sustain nodule function and activity (Evans, 1982).

Nodule senescence could partly explain the decline in N_2 fixation, particularly in the crown nodules after the mid pod-filling stage, as suggested by other workers (e.g., McDermott and Graham, 1989; Wolyn et al., 1989). During the period from mid pod-filling to physiological maturity, total dry weight of the nodules for the seed inoculation treatments declined, whereas that for the soil inoculation treatment accumulated 59.8 mg dry matter (Table 5.4). Notwithstanding the drop in N_2 fixation

in all the treatments, the granular-inoculant treatment accumulated 1.26 g plant dry matter during the last two growth phases compared to less than 0.43 g by the seed-inoculated treatments (Table 5.6). The nodulation in the non-inoculated control prevented definitive comparisons as to the extent to which low soil N limited growth, but it is apparent that available N limited plant growth in the seed inoculation compared to the soil inoculation.

It should be emphasized that in these studies, it was impossible to simulate field conditions. Although the pots used in this study permitted deeper soil depth, the pot size could restrict root activities and, therefore, become an influencing factor. It was evident from the present study, as well as from the field study with the same chickpea cultivar (Chapter 3), that root development was different under the two growth conditions. In the field, chickpea produced few, but thick and long, lateral roots which appeared suberized, whereas in the growth chamber it produced many lateral roots which appeared white, tender and spongy. The root morphology revealed in the field study was similar to other reports, which indicated that chickpea produces thick and long laterals with a low frequency of lateral branching (Mia et al., 1996, Rao and Ito, 1998). The root morphology and possibly anatomical change observed in the growth chamber might have been an adaptation to explore greater soil volume in order to exploit limited soil resources. It is well known that the morphological and anatomical differences in the component roots of a complex root system are related to their activity and functional differentiation (Yamauchi et al., 1996).

Nevertheless, it is clear that soil inoculation was superior to seed inoculation, particularly when the seed was inoculated with liquid-formulated inoculant. It is also evident that any inoculation strategy, such as inoculum placement, should be confirmed under field conditions.

6. EFFECT OF FUNGICIDE SEED TREATMENT ON RHIZOBIAL SURVIVAL AND NODULATION OF CHICKPEA

6.1 Introduction

Chickpea seeds are often treated with fungicides to prevent losses due to seed-borne pathogens and damping off. In addition, rhizobia are applied to the seeds to ensure effective nodulation and subsequent N_2 fixation. Although reports are conflicting, several studies have conclusively shown that some of these chemicals are incompatible with *Rhizobium* (e.g., Welty et al., 1988; Ramos and Ribeiro, 1993)

In an experiment on the survival of *B. japonicum* on chemically treated soybean seed, Revellin et al. (1993) found that Apron reduced viable rhizobia by 61% after one hour following seed inoculation. Similarly, Captan and pentachloronitrobenzene (PCNB) reduced viable *B. japonicum* by 18 and 78%, respectively, during a 1-h exposure (Curley and Burton, 1975). Graham et al. (1980), working with *R. phaseoli*, also observed that on seeds treated with Captan, less than 10% of the rhizobia survived after 24 h fungicide-rhizobia contact compared to more than 90% survival in a non-fungicide-treated control. The toxic effects of thiram on rhizobial survival have been reported (Graham et al., 1980; Tu, 1980; Hashem et al., 1997), but Curley and Burton (1975) found no adverse effect on the survival of *B. japonicum*.

In field studies, Captan adversely affected nodulation in inoculated chickpea (Thomas and Vyas, 1984; Welty et al., 1988), soybean (Graham et al., 1980; Chamber and Montes, 1982; Tesfai and Mallik, 1986) and pea (Rennie et al., 1985), but Rennie and Dubetz (1984) found no effect on soybean nodulation in a two-year field study, although shoot N yield at anthesis was reduced. Thomas and Vyas (1984) and Welty et al. (1988) observed no detrimental effect of thiram or metalaxyl on nodulation and yield in inoculated chickpea. On the contrary, Bhattacharyya and Sengupta (1984) found that seed treatment with thiram reduced nodulation 40 DAP in inoculated

chickpea. Revellin et al. (1993) similarly noted a significant decrease in nodulation and yield of soybean when the inoculated seeds were treated with Apron (metalaxyl). Similar harmful effects of thiram and metalaxyl application on nodulation were reported for inoculated pea and faba bean (Rennie et al., 1985). It was demonstrated that different species and strains of the same species of *Rhizobium* differed in their sensitivity toward various fungicides (Mallik and Tesfai, 1983). Thus, the compatibility of these chemicals with chickpea *Rhizobium* must be evaluated. The objective of this study was to examine the effect of four commercial fungicides, Apron®, Arrest 75W®, Crown®, and Captan on: 1) the survival of *Rhizobium ciceri* strain CP39 inoculated onto chickpea seeds; and 2) nodulation, nitrogen fixation, and dry matter production of inoculated chickpea in the growth chamber.

6.2 Materials and methods

6.2.1 Seed sterilization and treatment

Seeds of desi chickpea were surface sterilized by immersing the seeds for 3 min in 70% alcohol, followed by a 3 min treatment with 3% sodium hypochloride. The seeds were rinsed six times with sterile water, dried in a sterile laminar airflow hood and treated separately with one of the four fungicides at the manufacturers' recommended application rate. The formulation, active ingredients and the rate of application of the fungicides are listed in Table 6.1.

Table 6.1. List of fungicides used to treat chickpea seeds.

Treatment	Formulation	Active ingredient	Rate (per kg seed)
Apron®-FL	Powder	28.35% metalaxy [N 2,6-dimethylpheny) –N-(methoxyacetyl) alanine	2.5g
Arrest 75W®	Powder	50% thiram (tetramethyl thiuram disulfide) 20% carbathiin (5,6-dihydro 2-methyl-1,4 oxathiin-3-carboxanilide) 5% oxycarboxin (5,6-dihydro 2-methyl-1,4 oxathiin-3-carboxanilide 4, 4 -dioxide)	2.8g
Captan 50W†	Powder	50% N-[tri-chloromethylthio]-4-cyclohexene-1,2-dicarboximide	2.0g
Crown®	Liquid	92 g l^{-1} carbathiin (5,6-dihydro 2-methyl-1,4 oxathiin-3-carboxanilide) 58 g l^{-1} thiabendazole [2-(1,3-thiozol-4-yl) benzimidazole]	18ml

® Uniroyal Chemical Ltd

† United Agri Products, Dorchester, Ontario

The fungicide-treated seeds were stored for 7 days and then inoculated with peat-based inoculant containing *Rhizobium ciceri* strain CP39 (ICARDA, Aleppo, Syria; and kindly formulated by MicroBio RhizoGen, Saskatoon, SK) at the recommended rate of 1.95 g kg^{-1} seed, using 5 ml of 1% gum arabic solution as sticker to deliver approximately 10^5 *Rhizobium* cells $seed^{-1}$. The seeds were stored in sterile containers at 4°C in preparation for the survival experiment and for evaluation of chickpea growth in a controlled-environment. Non-fungicide treated seeds were also inoculated and stored as before.

6.2.2 Rhizobial survival on treated seeds

At 4, 12, 24 or 48 h after inoculation, 40 seeds from each fungicide treatment were removed and divided into four subsamples of 10 seeds each. Each subsample was transferred into test tubes containing 10 ml sterile water. The test tubes were shaken vigorously for 30 s to wash the inoculum off the seeds. One ml of the resultant suspension in each test tube was taken and serial dilution made from each subsample (Somasegaran and Hoben, 1994). Then, 0.1 ml of each dilution was plated by the spread-plate method on yeast extract-mannitol agar (YMA) (Vincent, 1970), containing Congo Red to aid in detecting contaminants. The YMA consisted of 1000

ml distilled water, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.1 g NaCl, 0.5 g $CaCO_3$, 0.5 g yeast extract, 15 g agar and 10.0 g mannitol and was adjusted to pH 6.8. The plates were incubated at 26°C and rhizobial colonies counted after 8 d. The experiment was repeated, using the same fungicides and inoculant.

6.2.3 Growth chamber study of nodulation and dry matter yield of chickpea

At each plating time (i.e., 4, 12, 24 or 48 h after inoculation), four seeds (from the seed sample stored for use in the rhizobial survival experiment) from each treatment were planted into a 2.5 L plastic pot containing a mixture of soil, sand and vermiculite in a 2:1:1 ratio (v/v). The soil was collected in August 1997 from a site 25 km east of Saskatoon, which had low mineral N levels and no history of chickpea production. After removing and discarding the top 3-cm layer, the soil was excavated to a depth of approximately 15 cm. The soil was dried and sieved using a 6-mm screen, before mixing with the required proportion of sand and vermiculite. Each pot contained about 3.5 kg soil mixture.

The pots were arranged in a randomized complete block design with four replications. The plants were grown in a controlled-environment cabinet (Model PGV 36, Controlled Environments Ltd, Winnipeg, MB) with 16-h daylength and a mean day and night temperature of 25 and 18°C, respectively. The relative humidity was maintained at 60 and 65%. The light source was composed of Cool White VHO and GRO-LUX VS VHO fluorescent lamps at a ratio of 3 to 1, supplying photosynthetically active radiation (PAR) of approximately 560 ±10 μmol m^{-2} s^{-1} at the top of the canopy. After emergence, the plants in each pot were thinned to two after which a 25 ml solution, containing 20 mg of 10.5% ^{15}N enriched $^{15}NH_4{}^{15}NO_3$, was applied to the surface of the soil in each pot. Flax was also grown as a reference crop in separate pots for the assessment of N_2 fixation by the ^{15}N-enrichment technique. The plants were watered on a daily basis with tap water to maintain field capacity, and at 14-d intervals with 100 ml half-strength N-free Hoagland nutrient solution (Hoagland and Arnon, 1938) per pot. A second experiment using seeds from the second rhizobial survival experiment was conducted under similar growth conditions.

6.2.4 Harvesting and plant tissue analysis

Harvesting was done at the late vegetative stage for the first experiment to assess dry matter yield. Nodulation was poor and was not assessed. For the second experiment, the plants were harvested at the flowering and early pod filling stages to examine nodulation and to determine dry matter yield. Nitrogen fixation was estimated on aboveground parts of the plants using the ^{15}N isotope dilution method as described in Section 5.2.4.

6.2.5 Statistical analysis

The plate counts for the two survival experiments were subjected to log transformation. The data were analyzed separately and the combined analyses performed, using the General Linear Model software developed by SAS Institute (1996). The fungicide treatments and fungicide-*Rhizobium* contact periods were considered fixed factors. In the combined analyses, experiments were considered random variables, whereas replications were nested within experiments. For the growth chamber study, data for all sampling times were analyzed separately. Like the rhizobial survival experiments, the fungicide treatments and the fungicide-*Rhizobium* contact periods before planting were considered fixed factors. Significant differences between treatment means were evaluated, using single degree of freedom contrasts (described previously in Section 5.2.5) at the 5% level of probability.

6.3 Results

6.3.1 Rhizobial survival on treated seeds

The survival of rhizobia on fungicide-treated seeds in the two experiments followed a similar trend, although the numbers that survived were higher in Experiment 1 as compared to Experiment 2 (Appendices 36 and 37). Whereas the decline in rhizobial numbers during the period between 4 and 24 h following inoculation was generally gradual in Experiment 1, it was drastic in Experiment 2. In both experiments rhizobial numbers stabilized between 24 and 48 h after inoculation, except for the decrease in the Captan treatment in Experiment 1 and the slight increase in the Arrest treatment in Experiment 2.

Averaged over experiments, fungicide treatments reduced the number of viable rhizobia on the chickpea seeds, although rhizobial survival on the non-fungicide control and the Crown treated seeds did not differ significantly (Table 6.2 and Fig. 6.1). Generally, Arrest, Apron and Captan reduced the numbers of rhizobia dramatically after 4 h of initial fungicide-*Rhizobium* contact as compared to the control. Both Apron and Captan significantly reduced the number of viable rhizobia even further during the 4 to 12 h contact period. In general, the toxicity of the fungicides increased in the following order: Control = Crown < Arrest = Apron < Captan.

Although the number of viable rhizobia recovered from inoculated seeds decreased with contact time (Fig. 6.1), the fungicide-*Rhizobium* contact period after the first 4 h had no significant effect on survival (Table 6.2). No significant interaction was observed between fungicide treatment and contact period. The significant experiment by fungicide interaction was due primarily to the differential response to the Arrest treatment in the two experiments (Appendices 36 and 37). The significant experiment x contact time interactions was due primarily to the lower recovery of viable rhizobial cells in Experiment 2 relative to Experiment 1.

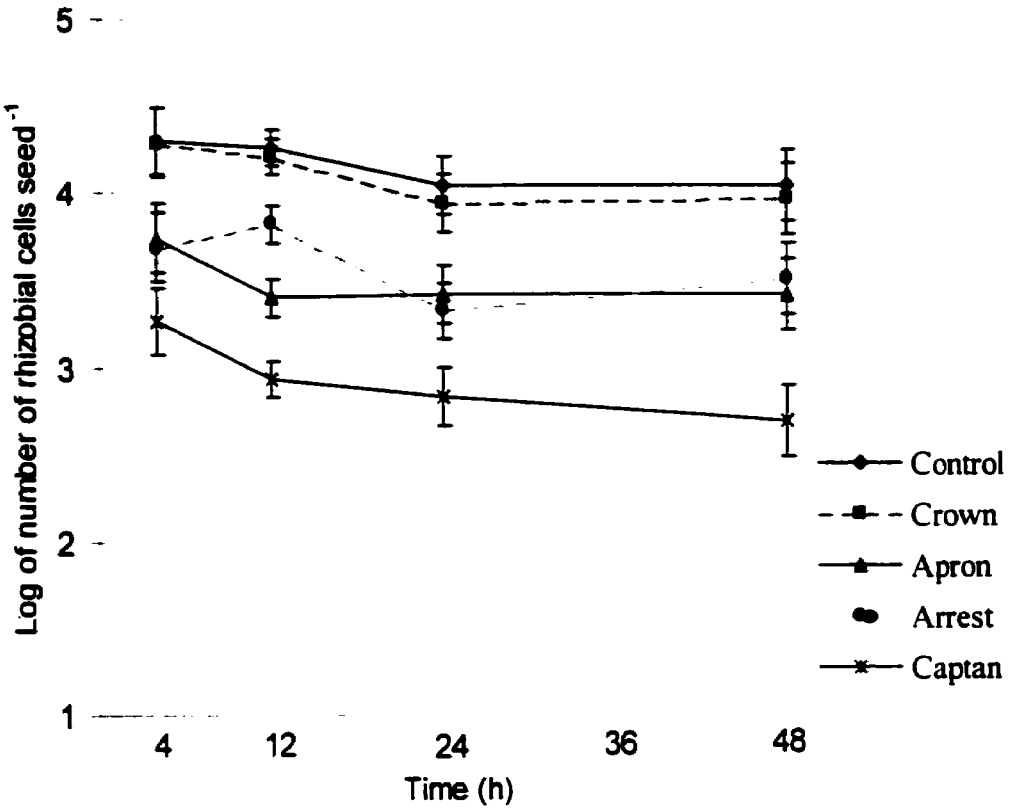

Fig. 6.1. Survival of *R. ciceri* strain CP39 on seeds treated separately with one of four fungicides seven days prior to inoculation as compared to the inoculated, but fungicide-free, control, combined over two experiments. Each point is the mean of eight replications, with vertical bars representing the standard error.

Table 6.2 Mean squares from the analysis of variance for the log-transformed data on viable rhizobia on chickpea seeds, combined over two experiments.

Source of variation	d.f.	Mean square
Experiments (Exp)	1	4.88
Replications in experiments	6	0.10
Fungicide treatment (F)	4	16.34*
Fungicide-*Rhizobium* contact time (T)	3	2.26
Exp x F	4	1.52**
Exp x T	3	0.92**
F x T	12	0.16
Exp x F x T	12	0.12**
Error	274	0.05
Total	319	

*, ** Significant at the 0.05 and 0.01 levels, respectively.

6.3.2 Nodulation, N_2 fixation and dry matter production

Nodulation in Experiment 1 was poor and was not assessed when the plants were harvested at the late vegetative stage. At this stage, seed treatment with Apron and Arrest had no significant effect on shoot dry weight, but Crown- and Captan-treated plants accumulated less biomass (Tables 6.3 and 6.4). Only Apron seed treatment reduced the proportion and amount of N_2 derived from fixation compared to the non-fungicide treated control.

In Experiment 2, the effect of fungicide treatment on nodulation was assessed at the flowering and early pod filling stages. At the flowering stage, the Crown, Apron and Captan seed treatments produced fewer nodules than the non-fungicide control but the Arrest treatment did not differ from the non-fungicide control (Tables 6.5 and 6.6). The Arrest seed treatment produced a higher nodule dry weight than the Crown or Captan seed treatment. Seed treatment with Crown significantly reduced shoot dry matter yield. At the early pod-filling stage, only the Captan seed treatment reduced the number of nodules relative to the non-fungicide control or the Apron treatment

(Tables 6.7 and 6.8). Contrasts between the non-fungicide control and the fungicide treatments individually showed that Crown and Arrest reduced nodule dry weight and shoot dry weight, whereas Captan reduced nodule dry weight only.

The %Ndfa at the flowering stage was not affected by fungicide treatment and ranged from 72.5% in the control treatment to 63.6% in the Captan treatment (Tables 6.5 and 6.6). However, the amount of N_2 fixed at this stage was significantly less for the seed treatment with Crown than for the control. At the early pod-filling stage all the fungicides, except Apron, significantly reduced %Ndfa and amount of N_2 fixed (Tables 6.7 and 6.8). Like the rhizobial survival experiment, the period of fungicide--*Rhizobium* contact after the initial 4-h exposure had no significant effect on number of nodules, nodule dry weight, shoot dry matter yield, %Ndfa or the amount of N_2 fixed in either experiment or at either sampling date (Tables 6.6 and 6.8). These results suggest that the major deleterious effects of the fungicides on rhizobial survival and plant growth occurred during the initial 4-h period of fungicide-*Rhizobium* contact. No fungicide x time interaction was detected for any of the parameters measured, indicating that the effects of the fungicides were similar for all the treatments over time.

Table 6.3. Dry matter production, percentage N derived from the atmosphere and amount of N_2 fixed at the late vegetative stage of chickpea plants grown from seeds treated with fungicide seven days prior to inoculation and the inoculated. but fungicide-free, control in Experiment 1.

Treatment	Shoot dry wt (g plant^{-1})	% Ndfa	N_2 fixed (mg plant^{-1})
Control	2.72	39.9	35.0
Crown	2.65	34.6	32.2
Apron	2.66	32.5	27.9
Arrest	2.68	37.5	34.2
Captan	2.62	35.6	31.6
$LSD_{(05)}$	0.06	5.9	6.7
Contrasts[†]			
Control vs. Crown	0.07*	5.3	2.8
Control vs. Apron	0.06	7.4**	7.1*
Control vs. Arrest	0.04	2.4	0.8
Control vs. Captan	0.10**	4.3	3.4

*, ** Significant at the 0.05 and 0.01 levels. respectively.
[†] Differences between specified treatments.

Table 6.4. Mean squares from the analysis of variance for dry matter production, percentage N derived from the atmosphere and N_2 fixed at the late vegetative stage of chickpea plants grown from seeds treated with fungicide seven days prior to inoculation and the inoculated, but fungicide-free, control in Experiment 1.

		Mean squares		
Source of variation	d.f.	Shoot dry wt	%Ndfa	N_2 fixed
Replications	3	0.105**	4512*	3916**
Fungicide treatment (F)	4	0.020*	130	123
Contrast				
Control vs. Crown	1	0.038*	231	62
Control vs. Apron	1	0.028	445**	403*
Control vs. Arrest	1	0.010	46	5
Control vs. Captan	1	0.070**	146	93
Fungicide-*Rhizobium* contact time (T)	3	0.013	129	138
F x T	12	0.010	40	76
Error	57	0.008	69	88
Total	79			

*, ** Significant at the 0.05 and 0.01 levels, respectively.

Table 6.5. Number of nodules, nodule dry weight, dry matter production, percentage N derived from the atmosphere and the amount of N_2 fixed at the flowering stage of chickpea plants grown from seeds treated with fungicide seven days prior to inoculation and the inoculated, but fungicide-free, control in Experiment 2.

Treatment	No. of nodules (plant^{-1})	Nodule dry wt (mg plant^{-1})	Shoot dry wt (g plant^{-1})	% Ndfa	N_2 fixed (mg plant^{-1})
Control	0.8	6.35	1.51	72.5	25.7
Crown	0.2	2.05	1.08	65.5	13.1
Apron	0.3	5.20	1.39	68.5	20.3
Arrest	0.7	11.05	1.43	70.3	25.0
Captan	0.3	2.55	1.28	63.6	18.9
$LSD_{(05)}$	0.4	6.20	0.41	ns	10.1
Contrasts[†]					
Control vs. Crown	0.6**	4.30	0.43*	7.0	12.6*
Control vs. Apron	0.5*	1.15	0.12	4.0	5.4
Control vs. Arrest	0.1	4.70	0.08	2.2	0.7
Control vs. Captan	0.5*	3.80	0.23	8.9	6.8

*, ** Significant at the 0.05 and 0.01 levels, respectively.
[†] Differences between specified treatments.

Table 6.6. Mean squares from the analysis of variance for number of nodules, nodule dry weight, dry matter production, percentage N derived from the atmosphere and N_2 fixed at the flowering stage of chickpea plants grown from seeds treated with fungicide seven days prior to inoculation and the inoculated, but fungicide-free, control in Experiment 2.

		Mean squares				
Source of variation[†]	d.f.	No. of nodules	Nodule dry wt	Shoot dry wt	%Ndfa	N_2 fixed
Replications	3	2.858**	428.5**	0.988*	263	425
Fungicide trt (F)	4	1.158*	208.5*	0.425	205	417
Contrasts						
Control vs. Crown	1	3.125**	149.3	1.463*	394	1259*
Control vs. Apron	1	2.000*	11.3	0.113	131	228
Control vs. Arrest	1	0.195	173.8	0.050	38	3
Control vs. Captan	1	2.258*	117.8	0.415	632	370
F-*R* contact time (T)	3	0.325	32.0	0.210	148	117
F x T	12	0.265	37.8	0.208	264	65
Error	57	0.420	77.0	0.340	310	204
Total	79					

*, ** Significant at the 0.05 and 0.01 levels, respectively.
[†] F-*R* = Fungicide-*Rhizobium*

Table 6.7. Nodulation, dry matter production, percentage N derived from the atmosphere and the amount of N_2 fixed at the early pod-filling stage of chickpea plants grown from seeds treated with fungicide seven days prior to inoculation and the inoculated, but fungicide-free, control in Experiment 2.

Treatment	No. of nodules (plant^{-1})	Nodule dry wt (mg plant^{-1})	Shoot dry wt (g plant^{-1})	% Ndfa	N_2 fixed (mg plant^{-1})
Control	6.1	176.6	2.50	78.5	41.8
Crown	3.2	113.1	1.76	70.2	25.6
Apron	6.8	122.9	2.26	73.0	32.2
Arrest	3.7	109.8	1.96	68.2	27.7
Captan	2.3	121.9	2.01	65.1	29.5
$LSD_{(05)}$	3.1	55.6	0.50	8.2	10.0
Contrasts[†]					
Control vs. Crown	2.9	63.5*	0.74**	8.3*	16.2**
Control vs. Apron	0.7	53.7	0.24	5.5	9.6
Control vs. Arrest	2.4	66.8*	0.54*	10.3*	14.1**
Control vs. Captan	3.8*	54.7*	0.49	13.4**	12.3*

*, ** Significant at the 0.05 and 0.01 levels, respectively.
[†] Differences between specified treatments.

Table 6.8. Mean squares from the analysis of variance for number of nodules, nodule dry weight, dry matter production, percentage N derived from the atmosphere and N_2 fixed at the early pod-filling stage of chickpea plants grown from seeds treated with fungicide seven days prior to inoculation and the inoculated, but fungicide-free, control in Experiment 2.

		Mean squares				
Source of variation[†]	d.f.	No. of nodules	Nodule dry wt	Shoot dry wt	%Ndfa	N_2 fixed
Replications	3	43.36	11592	1.36	603*	687*
Fungicide trt (F)	4	61.19*	11904	1.20	413*	641*
Contrasts						
Control vs. Crown	1	69.03	32261*	4.33**	537*	2096**
Control vs. Apron	1	4.50	23107	0.91	239	749
Control vs. Arrest	1	47.53	35718*	2.29*	839*	1608**
Control vs. Captan	1	114.38*	23931*	1.90	1437**	1223*
F-*R* contact time (T)	3	8.00	4023	0.45	122	155
F x T	12	35.62	5769	0.58	224	328
Error	57	19.31	6156	0.51	160	198
Total	79					

*, ** Significant at the 0.05 and 0.01 levels, respectively.
[†] F-*R* = Fungicide-*Rhizobium*.

6.4 Discussion

Rhizobia die rapidly on seeds following inoculation from exposure to adverse environmental conditions, such as excessive heat, dehydration and the presence of toxic substances (Kremer and Peterson, 1982; Griffith and Roughley, 1992; Hansen, 1994). The slow rate of decline in viable rhizobia in the inoculated, fungicide-free treatment 4 h after inoculation indicates that the decline in rhizobial survival was due primarily to the toxicity of the various fungicides. The treated and inoculated seeds were kept at 4°C, an optimum temperature for inoculant storage. At this temperature dehydration also was minimal. The high recovery of viable *R. ciceri* from seeds treated with Crown fungicide could possibly be that more *Rhizobium* inoculant adhered to the seed coat since it was the only fungicide applied in liquid formulation which contained additional adhesive. Although all treatments, including the Crown-treated seeds, were dried prior to inoculation, the gum arabic included in the formulation remained on the seeds and additional sticker solution used during the inoculation process probably made it stickier than the other treatments. Another possible explanation for the minimal effect of Crown on rhizobial viability could be that *R. ciceri* strain BCF 32 is tolerant to carbathiin and thiabendazole, the active ingredients in Crown.

The decline in the number of viable *R. ciceri* with Apron fungicide treatment agrees with the results of Revellin et al. (1993), who observed a sharp decline in the survival of *B. japonicum* on soybean seeds during a 24-h exposure to the fungicide. In contrast to this observation, Diatloff (1986) and Edmisten et al. (1988) found no adverse effect of Apron (metalaxyl) on the viability of *B. japonicum* and *R. meliloti* when fungicide-treated soybean and alfalfa seeds, respectively, were inoculated. The discrepancy in the results, reported by the various authors, may be due to the concentrations of the fungicides used, *Rhizobium* strain or the methods by which the inoculant was applied. For example, the product used by Diatloff (1986) contained 25% active ingredient, whereas that used by Revellin et al. (1993) was 35% as compared to 28.35% in the present study. Although the rate of fungicide application was not specified by Diatloff (1986), it is likely the rate was lower compared to that used by Revellin and coworkers which was double the rate used in the present study.

Also, in the study reported by Diatloff (1986), the soybean seeds were first inoculated and allowed to dry prior to fungicide treatment. Revellin et al. (1993) argued that with this method the fungicide-*Rhizobium* contact would not be very intimate, due to the absence of moisture as compared to a situation where fungicide-treated seeds were inoculated with peat inoculant slurry. Inconsistencies among results of various researchers could also arise because of considerable differences in tolerance among species and strains of rhizobia to different fungicides, as reported by several investigators (Faizah et al., 1980; Mallik and Tesfai, 1983).

The rapid loss of viability due to Captan exposure is consistent with previous reports of deleterious effects of this chemical on rhizobia, including *B. japonicum* (e.g., Curley and Burton, 1975; Mallik and Tesfai, 1983), *R. phaseoli* (Graham et al., 1980) and peanut *Bradyrhizobium* sp. (Hashem et al., 1997). Arrest (thiram + carbathiin + oxycarboxin) showed a limited toxicity which supports the findings of others workers (e.g., Graham et al., 1980; Revellin et al., 1993, Hashem et al., 1997). In contrast, Curley and Burton (1975) found no adverse effect of thiram on *B. japonicum* on soybean seeds.

According to the evaluation of toxicity, using the standard plate counts one might conclude that Crown is compatible with *R. ciceri*, but evaluation based on subsequent nodulation and dry matter yield data suggest differently. Although Apron was toxic to rhizobial survival, it did not affect nodule dry weight and dry matter production at the early pod-filling stage as evaluated in Experiment 2. However, the chemical inhibited nodulation when evaluated at the flowering stage and reduced %Ndfa and amount of N_2 fixed at the late vegetative stage. In field experiments, Castro et al. (1997), working with the fungicide mancozeb, also observed a significant decrease in dry weight of peanut plants at the R1 and R6 phenological stages compared to the non-fungicide treated control, but this difference disappeared by the final harvest. Other workers also reported the temporary effect of fungicides on nodulation, N_2 fixation and dry matter yield in soybean (Tu, 1977; Widin and Kennedy, 1983) and chickpea (Bhattacharyya and Sengupta, 1984). The trend observed in the present study suggests that the toxicity of Apron may have persisted in the soil for only a short time period, after which the remaining viable cells rapidly

multiplied and resulted in increased nodulation. This is possible because the soil environment can act as a buffer, reducing the potentially toxic effect by dilution of this chemical (Tu, 1977; Castro et al., 1997). In addition, the inoculant strains may have migrated away from the toxic zones (Alexander, 1961), reducing the effect of the chemicals on the chickpea-*Rhizobium* symbiosis.

When evaluated at the late vegetative stage, Apron seed treatment significantly decreased N_2 fixation, as determined by the ^{15}N isotope dilution technique, but this was not reflected in dry matter yield, indicating that the soil provided sufficient N (Table 6.3). On the other hand, the significant decline in shoot dry matter at the vegetative stage due to Captan seed treatment in the first experiment also indicated that the relatively high N_2 fixation was not translated into dry matter yield.

None of the fungicide seed-treatments had a significant influence on %Ndfa in Experiment 2 when evaluated at the flowering stage but Crown seed treatment reduced the amount of N_2 fixed (Table 6.5). However, by the early pod-filling stage (Table 6.7), the proportions and amounts of N_2 fixed for all the fungicide treatments, except Apron, were lower than for the non-fungicide treated control (Tables 6.5 and 6.7). Crown and Arrest seed treatments also reduced shoot dry matter production. The lack of any detrimental effects from the Apron seed treatment supports reports by several authors, who found that seed treatment with metalaxyl had no detrimental effect on nodulation and N_2 fixation (Rennie et al., 1985; Diatloff, 1986; Edmisten et al., 1988). In contrast, others have reported that Apron decreased nodulation (Revellin et al., 1993; Hashem et al., 1997), resulting in a significant reduction in shoot dry matter, plant N content (Hashem et al., 1997) and seed yield (Revellin et al., 1993). Similarly, the reduced nodulation and N_2 fixation from Captan treatment are in agreement with previous reports (Graham et al., 1980; Chamber and Montes, 1982; Thomas and Vyas, 1984; Rennie et al., 1985; Tesfai and Mallik, 1986; Welty et al., 1988; Hashem et al., 1997). However, the effect of Captan on shoot dry weight at the early pod-filling stage contradicts results of Hashem et al. (1997), who reported a significant reduction in shoot weight of peanut plants due to Captan treatment. Graham et al. (1980) reported that the main effect of Captan was to reduce the survival of seed-applied rhizobia in contact with it.

Arrest, as a seed coat dressing, had a limited toxicity effect on the viability of *R. ciceri*, but in the growth chamber, nodulation, shoot dry matter and N_2 fixation were reduced at the early pod-filling stage. Similarly, Crown had no influence on rhizobial viability 48 h prior to planting, but significantly reduced nodulation, shoot dry weight and N_2 fixation when evaluated in the growth chamber experiment. These results suggest that the correlation of viable counts to nodulation, N_2 fixation or yield may be unreliable because rhizobia can lose their ability to induce nodulation before they lose their ability to multiply (Curley and Burton, 1975). Although Crown did not affect the viability of *R. ciceri*, it may have had a negative impact on some functional aspect of the rhizobial cells that subsequently reduced their ability to nodulate the plant roots. Other authors have also reported contradictory results between laboratory evaluation of fungicides and field performance. For example, Curley and Burton (1975) found that Captan and Carboxiin were not harmful to *B. japonicum* survival, but in field studies, these chemicals reduced nodulation. Similarly, peanut seed treated with Vitavax did not affect the viable number of rhizobial strain USDA 3456, but it severely reduced nodule mass, shoot dry weight and plant N content (Hashem et al., 1997). These findings demonstrate that the viability test alone only provides a partial measure of compatibility and must be correlated with growth chamber or field data.

Nevertheless, the data for Arrest (50% thiram) is consistent with previously published reports. Tu (1981) found that thiram reduced soybean nodule mass and acetylene reduction activity in a greenhouse study. In greenhouse and field studies, Hashem et al. (1997) also observed that seed treatment with thiram significantly reduced nodule formation, shoot dry matter, plant N content and seed yield. However, in field studies with chickpea, seed treatment with thiram increased nodulation and seed yield (Thomas and Vyas, 1984; Wetty et al., 1988). It must be noted that the studies, which reported beneficial effects of thiram as a seed coating relative to the non-fungicide control, were conducted on fields heavily infested with *Pythium*. Hence, the higher seed yield was primarily due to the increase in plant stand. Indeed, this does not indicate whether the fungicide was harmful or compatible with the inoculant strains. If the fungicide was detrimental to rhizobial survival or effectiveness, any advantage for not treating the seeds may have been masked by the destruction caused

by the pathogen. It is also possible that thiram may have reduced the competition between the inoculant rhizobia and other soil organisms, resulting in increased nodulation.

The present study indicated that the toxic effect of the fungicides on the survival of rhizobia on seeds increased with contact time (Fig. 6.1; Appendices 37 and 38), although the numbers from the standard plate counts did not differ significantly among the contact times (i.e., 4, 12, 24 or 48 h) (Table 6.2). This corresponded well with nodule numbers and dry weight as well as other parameters, such as plant dry weight, %Ndfa and N_2 fixed in the growth chamber (Tables 6.4, 6.6 and 6.8). Curley and Burton (1975) also reported no significant differences in nodule numbers evaluated on 2-wk old plants grown from thiram-, Captan- or PCNB-treated soybean seeds planted 1, 4 or 24 h after inoculation. Similarly, Revellin et al. (1993) found no significant differences in *B. japonicum* survival or soybean nodulation after a 1 or 4-h exposure of *Bradyrhizobium* to five fungicides, including Apron, when assessed in the greenhouse 28 DAP. However, in contrast to the present study, this report indicated significant deleterious effects of the fungicides between 4 and 24 h of contact. The discrepancy could be attributed to the storage conditions of the fungicide-treated seeds after inoculation and prior to the survival tests and planting. For example, in the present study the seeds were stored at 4°C, whereas the seeds used by Revellin et al. (1993) were stored at 20°C. Despite the disagreements between the results of this study and others, the results highlight the fact that fungicide-treated seeds should be sown as soon as possible after inoculation.

The discrepancies between the present study and previous reports and the contradictions in the literature indicate the complexity of the subject. Hence, care must be taken in the interpretation of such results. The respective effect of each fungicide will probably depend on the *Rhizobium* species or strain. *Rhizobium*-fungicide contact period prior to planting, concentration of the fungicide and the environmental variables. This highlights the importance for examination and selection of fungicides for a specific *Rhizobium* strain.

Although the length of time the *Rhizobium* were exposed to the fungicide before planting had no influence in the present study because of the conditions of

storage, others (e.g. Curley and Burton, 1975; Graham et al., 1980) have demonstrated its importance in assessing compatibility. Hence, when chemically-treated seeds are inoculated, they must be planted immediately in the field to minimize the effect of the chemical on the inoculum.

Rhizobium strains display different sensitivities to different fungicides (Mallik and Tesfai, 1983) and the tolerances of strains differ with regard to their compatibility with fungicides (Tesfai and Mallik. 1986). Odeyemi and Alexander (1977) reported that thiram-resistant strains of *R. meliloti*, in the presence of thiram, enhanced nodulation, dry weight and N content of plants compared to the treatment in which the inoculant strain was not resistant to the fungicide. This area certainly needs further studies in order to develop fungicide-resistant strains for use as chickpea inoculants.

Another approach to overcome the harmful effects of fungicides is to adopt an alternative method of inoculation which avoids direct fungicide-*Rhizobium* contact. Granular inoculant, applied to the soil, avoids intimate contact with the fungicide and has been effective in some studies (Brockwell et al., 1980; Graham et al., 1980; Ramos and Ribeiro, 1993). A granular inoculant could be useful in kabuli chickpea production in Saskatchewan because fungicide seed-treatment is required. Therefore, further research is needed to examine the use of granular inoculant in combination with the fungicides tested in the present studies in field-grown chickpea. Because laboratory or growth chamber conditions do not precisely reflect the conditions in the field, it is suggested that the present experiment be repeated in the field to confirm the results.

7. GENERAL DISCUSSION

A growing awareness of the benefits of including pulses in rotations in Saskatchewan has created interest in growing new pulse crops, including chickpea, in the Dark Brown and Brown soil zones (Vandenberg and Slinkard, 1996). The compelling need to exploit the N_2-fixing potential of these leguminous crops has focused attention on *Rhizobium* inoculation technologies. Until recently, most of the legume inoculants available on the market were formulated as liquids or peat-based powders that are applied to the seed before planting. However, granular inoculants with peat- or clay-based carrier materials have been introduced recently. The granular inoculants are applied to the soil and have given good results as compared to the seed-applied inoculants in some studies (Scudder, 1975; Dean and Clark, 1977; Bezdicek et al., 1978; Muldoon et al., 1980: Hardarson et al., 1989). This study was undertaken to assess granular inoculants for chickpea with special interest in inoculant placement and its effects on nodule distribution and the time course of N_2 fixation.

Chickpea was chosen for the study for two reasons. Firstly, being a new crop in Saskatchewan, the soil is free of indigenous chickpea rhizobial strains resulting from previous inoculations. Secondly, the chickpea-*Rhizobium* symbiosis is highly specific (Gaur and Sen, 1979; Silsbury, 1989), and should prevent cross nodulation in the presence of other resident rhizobia. Many indigenous rhizobia are ineffective in N_2 fixation, but outcompete the introduced strain in nodule formation (Zdor and Pueppke, 1990). Hence, the presence or absence of a native rhizobial population in a field can affect inoculation success. Thus, chickpea in Saskatchewan provides an excellent model to examine response to inoculation because the confounding effects of indigenous rhizobia are necessarily minimized. Both desi and kabuli chickpeas were used in the field studies (Chapter 3), but only desi chickpea was used in the growth chamber experiments (Chapters 5 and 6) because both chickpea types responded similarly to inoculation treatments and rhizobial strain combinations in the field. In addition, no rhizobial strain interactions were evident for any of the traits, such as

nodule dry weight, shoot dry weight, plant biomass and seed yield. Desi chickpea was selected for the growth chamber studies because it is less susceptible to insects and diseases than the kabuli chickpea (Smartt, 1990; Singh, 1991; Saskatchewan Pulse Crop Development Board, 1997).

The method of inoculation had a marked influence on the nodulation, N_2 fixation and yield of chickpea. In field (Chapter 3) and growth chamber studies (Chapter 5), the liquid-formulated inoculants were inferior to the peat-based and the granular inoculants in all traits. Bissonnette and Lalande (1988) observed that the carrier material for the inoculum affected the survival of the rhizobia during stress, suggesting that the rhizobial strains in the liquid inoculants were much more exposed to unfavourable stresses after inoculation onto the seed than those on the peat-based inoculants. Although both the liquid and the peat-based inoculants were applied to the seed, data on nodule numbers and dry weight indicate that the peat allowed rhizobia to survive on the seeds to a greater extent than the liquid inoculant. Rice et al. (1998) stated that rhizobia in a granular inoculant can multiply after planting whereas viable rhizobia in a peat or liquid inoculant on the seed decline after seeding. Zdor and Pueppke (1990), working with liquid and peat inoculant carriers, indicated that a peat formulation may help protect the rhizobial strains from antagonistic components that would reduce their populations. Thus, a peat carrier, in contrast to a liquid carrier, may increase strain survival by reducing desiccation or heat stress of the cells, a major factor involved in the establishment of rhizobia in soil (Hansen, 1994).

Although total nodule number and dry matter yield data for the peat-based inoculants generally did not differ significantly from that for the granular inoculants in the present study, several workers have recognized many limitations associated with seed inoculation. For example, Roughley et al. (1993), using peat-based inoculant, reported that 95% of the *Bradyrhizobium*, originally present in the inoculant applied to lupin seed, died during inoculation and sowing, due to desiccation. Brockwell et al. (1988) also observed that substantial losses of inoculum viability of up to 99.9% occurred between inoculation and sowing when soybean seed was inoculated with peat-based inoculants, partly due to separation of the inoculant and the seed, as it passed through the machinery.

Other factors, such as pesticide seed treatment, adversely affect nodulation in inoculated chickpea (Bhattacharyya and Sengupta, 1984; Thomas and Vyas, 1984; Welty et al., 1988). The results of the growth chamber study (Chapter 6) confirmed these reports, suggesting that treating the seeds used for the field experiments (Chapter 3) with Apron and Crown may have reduced nodulation in the seed-applied inoculant treatments. The impact of the fungicide likely was greatest for the treatments in which the seeds were inoculated with liquid inoculants. The study on the effect of fungicide-*Rhizobium* interactions also revealed three important facts. Firstly, some fungicides may directly affect the number of viable rhizobia inoculated onto the seed, but may not affect nodulation, N_2 fixation or plant growth significantly, as observed when the seeds were treated with Apron. In such instances, the inhibitory effect may be apparent during the early growth stages of the plant, but disappear during the later part of the growth cycle. Secondly, some fungicides, such as Crown, may not have an obvious effect on the number of viable rhizobia on the seed, but may severely reduce nodule number and dry weight, N_2 fixation and plant dry matter production. Presumably, the ability of the rhizobia to nodulate decreased on contact with the fungicide, even though the cells survived and were recovered in the viability test. The results suggest that the viability test must be correlated with growth chamber or field data in order to have a reliable measure of fungicide-*Rhizobium* compatibility. Thirdly, the number of viable rhizobia on the seed in each of the fungicide treatments dropped drastically in the first four hours and continued to decline with the length of time the rhizobia were exposed to the fungicide. Although the decline after the initial 4 h of fungicide-*Rhizobium* contact was not significant, these results suggest that fungicide-treated seeds should be planted as soon as possible after inoculation.

Although the sensitivity of different rhizobial strains to the various fungicides was not assessed, several investigators (e.g., Mallik and Tesfai, 1983; Hashem et al., 1997) demonstrated that different species and strains of the same species of *Rhizobium* differed in their sensitivity toward various fungicides. This evidence suggests that the compatibility of each specific fungicide-*Rhizobium* combination must be evaluated. In a review, Howieson (1995) suggested five strategies to overcome fungicide-*Rhizobium* incompatibility: 1) selection of fungicide-tolerant rhizobial strains; 2) selection of

persistent strains of rhizobia to avoid repeated inoculation; 3) selection of legume cultivars which are resistant or tolerant to diseases; 4) the use of spray inoculation (liquid inoculant sprayed directly into the soil); and 5) the use of seed coating materials which physically separate the rhizobia from the fungicide. Although some of these strategies have been studied, they have had limited success, emphasizing the need for a critical look at the use of granular inoculants which can be applied to the soil below the seed, thereby, limiting the impact of the fungicide on nodulation and N_2 fixation.

Although total nodule number and dry weight data for the granular inoculant generally were not statistically different from that for the peat-based inoculant (Chapters 3 and 5), the fundamental difference in nodulation between seed-applied inoculants (liquid and peat-based) and soil-applied inoculant (granular) was the distribution of the nodules on the root system. The granular inoculants, particularly, those placed below the seed, produced most of their nodules on the lateral roots in the lower part of the root system. In contrast, the seed-applied inoculants formed nodules predominantly at the crown region of the root system. This finding supports previous reports (Danso and Bowen, 1989; Hardarson et al., 1989; Wadisirisuk et al., 1989; Danso et al., 1990) and suggest that, the position of the nodules on the root system depended to a large extent on the depth of inoculum placement. The data for the granular inoculant also indicated that in addition to crown nodulation, inoculation of the soil at the seeding depth enhanced lateral root nodulation. This contradicts the view that earlier-formed tap and crown nodules suppress nodulation on the younger roots, at the lower part of the root system (Kosslak and Bohlool, 1984; George et al., 1992). The fewer nodules formed on the lateral roots by the seed-applied inoculant may be associated with the restriction of the rhizobia to the vicinity of the seed and the inability of the rhizobia to contact the younger roots at the lower part of the root system. Rhizobia movement in the soil is restricted (Madsen and Alexander, 1982; Chamblee and Warren, 1990; Worrall and Roughley, 1991; Issa et al., 1993a,b); hence, the inoculum should be strategically placed to colonize the rhizosphere and to form nodules as the roots extend out and down the soil profile.

A consequence of placing granular inoculant below the seed is delayed nodulation, although more nodules may form as the plant ages and the root system becomes more extensive (Bhuvaneswari et al., 1981; Brockwell et al., 1988). In contrast, seed-applied inoculation induces early nodulation, which may increase only slightly during the later part of the growth cycle. This was particularly evident in the growth chamber study (Chapter 5) because nodule evaluation began relatively early, during the vegetative stage (28 DAP) and was performed at two-week intervals, until physiological maturity. The dry weight of the lateral root nodules increased steadily over the growing season and the increase was greater than for the crown nodules. Similar observations were reported for soybean (Brockwell et al., 1988) and cowpea (Kahn and Stoffela, 1991).

Likewise, a pattern similar to that for nodule dry weight has been reported for N_2 fixation, measured as acetylene reduction activity or by the ^{15}N isotope technique (Hardarson et al., 1989; McDermott and Graham, 1989; Wolyn et al., 1989; Danso et al., 1990). In the present study, N_2 fixation was assessed by the ^{15}N isotopic enrichment and ^{15}N natural abundance techniques. The growth chamber study, using the ^{15}N isotope dilution technique (Chapter 5), indicated that the %Ndfa and N_2 fixed for the granular and peat-based inoculants did not differ from the late vegetative stage to physiological maturity. However, at the late vegetative stage, the granular-inoculated plants derived the same proportion and amount of N from fixation as the plants grown from seeds inoculated with the peat-based inoculants, but then soil inoculation resulted in a slightly greater %Ndfa and N_2 fixed than seed inoculation until physiological maturity. This may be an indication that the lateral root nodules were increasingly more active after the late vegetative stage in comparison to the crown nodules, which were predominantly formed by the seed-applied inoculants. However, the magnitude of the increase in N_2 fixation in the soil-inoculation treatments was low and could be due to sufficient available soil N levels (Hardarson et al., 1989) and the good growing conditions in the growth chamber. Brockwell et al. (1988) concluded that neither inoculation procedure was better than the other, and that any observed superiority of either was the result of environmental conditions at planting and during plant growth.

For the field experiments, N_2 fixation was assessed for the harvested chickpea seed using the ^{15}N natural abundance method. An important advantage of this method is that it required no ^{15}N-labelled fertilizer application, and time-consuming fieldwork was avoided, making it relatively inexpensive. However, it was necessary to establish the magnitude of isotopic fractionation during N_2 fixation for the part of the plant sampled (Steele et al., 1983; Bergensen et al., 1986; Ledgard, 1989). Furthermore, both the host plant and the rhizobial strain can influence the isotopic fractionation value (Bergensen et al., 1986; Yoneyama et al., 1986; Ledgard, 1989). Hence, a hydroponic experiment was conducted in a growth chamber, using desi and kabuli chickpeas inoculated with the same rhizobial strains used in the field studies (Chapter 4). For the desi chickpea, the isotopic fractionation (β) value was higher for the single strain CP39 than for the mixed strains (27A2, 27A7 and 27A9). Thus, %Ndfa for the seed and the amount of seed N fixed would have been under-estimated, if the (β) value for the single strain had been used in calculations on plants inoculated with the mixed strains. In contrast, the isotopic fractionation values for the kabuli chickpea and rhizobial strain combinations were similar, indicating that an accurate estimate of the proportion and amount of N fixed for the seed would have been obtained from any of the values. The results indicated that %Ndfa for the seed and the amount of seed N fixed were generally greater for soil inoculation than for seed inoculation, supporting previous reports (Muldoon et al., 1980; Dubetz et al., 1983). The environmental conditions in the field were variable as compared to those in the growth chamber; hence, it is not surprising that the differences in %Ndfa between the seed and soil inoculations were large from the field data than from the growth chamber data. Moreover, this substantiates the conclusion that it is the environment that dictates the differences in inoculation response (Brockwell et al., 1988).

The N_2 fixation data from the different experiments (Chapters 3, 5 and 6) varied, presumably due in part to differences in environmental variables under which the plants were grown and method of measurement. Nevertheless, these studies (Chapters 3 and 5) indicated that the lateral root nodules made an important contribution to N_2 fixation, particularly during the reproductive stages. For the field study, the relationship between nodulation pattern and N_2 fixation was assessed

indirectly by correlating the dry weight of crown or lateral root nodules to plant dry matter at the flowering, early pod-filling and late pod-filling stages, and also to seed yield. The results revealed that the dry weight of the lateral root nodules was positively correlated with dry matter yield and seed yield, consistent with the data from the growth chamber study (Chapter 5) and data of others (McDermott and Graham, 1989; Wolyn et al., 1989; Danso et al., 1990; Vikman and Vessey, 1992, 1993; Hardarson, 1993). The relationship was further substantiated by data on shoot dry matter at flowering in the desi field experiment, where soil inoculation was significantly greater than the seed inoculation at the 5% level, but the differences increased to the 1% level at the early pod-filling stage. For the growth chamber experiment (Chapter 5), the plant dry matter accumulation pattern was similar to the N_2 fixation pattern, and most importantly, the soil-inoculation treatment accumulated a greater plant biomass during the later part of the growth cycle than the seed-inoculation treatments. Previously, the N_2-fixing potential of nodules on the lateral roots and the lower part of the root system had been disregarded and considered less important than the nodules formed at the crown or on the top-most part of the root system (Hardarson, 1993). The results of the present study highlight the need for careful consideration of the nodules on the lateral roots or at the lower part of the root system in N_2 fixation assessment, either by nodule rating or acetylene reduction assay. These nodules usually fall off during excavation of the field-grown plants or often are not sampled.

Generally, the results of these studies indicate that the greater yields achieved from the granular inoculants were due to the preponderance of relatively young lateral root nodules which maintained activity during the later part of the growing season. This was particularly evident when the granular inoculant was placed below the seed. In 1998, the correlation between dry weight of the lateral root nodules and seed yield for the kabuli chickpea experiments was poor, due primarily to the delayed germination and reduced plant stand as a result of the severe drought. In this case, the hard soil surface from the drought, coupled with the additional opener for deep placement of the granular inoculant, increased resistance of the soil to penetration and resulted in shallow planting. As a consequence, the seed was placed in a layer too dry

for optimum germination and emergence. Although the plants in these treatments, which germinated later, were comparable with those in the other treatments, the delayed germination reduced the growth period for optimum yield. In Saskatchewan, the growing season is relatively short and sometimes exacerbated by terminal drought as in 1997 and 1998. Thus, any delay in plant establishment likely will reduce the length of time for N_2 fixation, pod-filling and seed maturation. Even in this situation, where yields were not increased by granular inoculants, the seed protein concentration was enhanced as in the other granular inoculant treatments, in comparison to that for the seed-applied inoculants.

In a year with unfavourable weather conditions, placing granular inoculant below the seed without affecting the seeding depth may be superior to seed inoculation. Normal seeding depth into good moisture should minimize temperature fluctuation in that soil zone, both of which are important for rhizobial survival and nodule formation. Alternatively, when environmental conditions are good during and after planting, seed or soil inoculation is equally likely to establish a successful symbiosis (Brockwell et al., 1988). However, where the seed-applied inoculum fails to form nodules on the lower part of the root system, i.e., on the lateral roots, due to the limited migration of the inoculant strain, soil inoculation may enhance N_2 fixation and improve yield and seed quality.

The nodules on the lateral roots or lower part of the root systems are young and more active than the crown nodules during pod-filling (McDermott and Graham, 1989; Wolyn et al., 1989). During this growth phase, the soil mineral N levels are usually depleted, reducing the N uptake rate (Imsande, 1989; Vessey, 1992). Thus, the nodules formed on the lower or lateral roots contribute significant amounts of fixed N to the plant during seed formation.

With the appropriate seeding equipment, chickpea and other legumes grown in the Brown and Dark Brown soil zones in Saskatchewan could benefit from soil inoculation. In cases where yield responses are not observed, N concentration in the grains or plant parts may increase over that from seed-applied inoculants.

8. SUMMARY AND CONCLUSIONS

The depth of inoculum placement significantly influenced the position of the nodules on the root system. The granular inoculants, in particular, when placed below the seed, formed nodules mainly on the lateral roots, whereas the nodules produced by the seed-applied inoculants (liquid and peat-based) were located predominantly at the crown region. The total number of nodules in all treatments was not always consistent with total nodule dry weight, but based on dry weight, the liquid inoculant was generally inferior to the peat or the granular inoculants.

Treating the seed with fungicide influenced nodulation by decreasing the number of viable rhizobia on the seed. Seed treatment with Crown fungicide did not affect survival of the rhizobia, but reduced nodule dry weight, %Ndfa, amount of N_2 fixed and dry matter yield. Arrest, Apron and Captan were harmful to rhizobial survival on the seed with Captan being the most toxic. However, the inhibitory effect of these fungicides was not obvious when evaluated at the late vegetative and flowering stages, except for the lower shoot dry matter and N_2 fixation for the Captan and Apron treatments, respectively, at the late vegetative stage.

At the early pod-filling stage, Arrest and Captan reduced nodule dry weight, %Ndfa and N_2 fixed, but only Arrest reduced shoot dry matter production. Seed treatment with Apron was not detrimental to the chickpea-*Rhizobium* symbiosis at the early pod-filling stage. The inconsistency between the standard plate count and the growth chamber study reveals that a reliable measure of fungicide-*Rhizobium* compatibility must involve both a viability test and growth chamber or field data. The most important information revealed by the study was that the major deleterious effect of the fungicides on rhizobial survival and plant growth occurred during the initial 4-h period of fungicide-*Rhizobium* contact. Therefore, when rhizobia are inoculated onto seed they must be planted immediately to reduce the effect of environmental variables including seed-treated fungicides.

In the field and in the controlled-environment studies, nodule formation was delayed in treatments where the granular inoculant was placed below the seed, due to the time lag that occurred before the growing root contacted the inoculant rhizobia. Total nodule dry weight for all treatments in the growth chamber experiments increased to a peak at the late pod-filling stage and then declined. However, the granular-inoculant treatment accumulated greater nodule dry matter after the mid pod-filling stage than the seed-applied treatments.

The position of the nodules (associated with the age of nodules), rather than the weight of the nodules, influenced the yield parameters. In the field study, the dry weight of the lateral root nodules was positively correlated with plant dry matter on an individual plant basis at the flowering, early pod-filling and late pod-filling stages. Similarly, the dry weight of the lateral root nodules was positively correlated with seed yield. In contrast, the relationships between these traits and dry weight of the crown nodules were weak. However, the shallow seeding depth for the granular inoculant placed below the seed in 1998, due to the hard soil surface, diminished the relationship between the lateral root nodules and the seed yield in the kabuli chickpea in that year. Thus, inoculating the soil with granular inoculant was superior to seed inoculation with either peat or liquid inoculants in plant dry matter production and seed yield, although this was not the case when the efficiency of the seeding equipment was affected by the soil conditions. Furthermore, granular inoculants placed below the seed were better than granular inoculants placed in the seed furrow in 1997, but not in 1998, when shallow planting occurred in the treatment where the granular inoculant was placed below the seed.

In the controlled environment, the differences in dry matter production, %Ndfa and N_2 fixed among the inoculated treatments were not significant, except for a few differences that occurred, particularly between the liquid and the granular inoculants. However, these parameters were generally greater for the granular inoculant and like the nodule dry weight data, the granular inoculant treatment accumulated a substantial portion of its dry matter during the later part of the growth cycle as compared to the seed-applied inoculant treatments. The %Ndfa increased progressively from the late vegetative stage to a maximum at the mid-pod filling stage, but the highest fixation

rate occurred between the flowering and the early pod-filling stages with little or no N_2-fixing activity thereafter until physiological maturity. In the field the granular and peat-based inoculants resulted in higher seed protein concentration, %Ndfa for the seed and amount of seed N fixed compared to the liquid inoculant. The field and growth chamber data indicate that the peat and granular inoculants are equally effective in establishing successful symbiosis when environmental conditions are not limiting.

Notwithstanding the limited yield advantage of soil inoculation over seed inoculation, inoculating the soil 2.5 to 8 cm below the seed will be more beneficial than inoculating the seed. In that soil zone, the inoculant strains are placed in a more conducive environment, and physically separated from seed-treated pesticides. The young growing roots of the host plant are more likely to encounter the inoculant strains at that soil depth for infection and subsequent nodule formation. These later-formed nodules may be important in supplying fixed N_2 to the plant at a period when the N requirement is at its maximum.

Suggested areas for future research

1. The inability of the inoculant strain to move with the developing root system is a major factor limiting nodulation and N_2 fixation. Therefore, future studies should include selection of inoculant strains which are more motile to enhance nodulation on the entire root system of the host and not just the crown area.

2. The greater, but limited, yield advantage for the granular inoculant may be associated with the inoculum application rate. Therefore, field studies using higher than recommended rates should be evaluated, particularly in first-time fields.

3. Accurate placement of both seed and inoculum into moist soil is essential for establishing an effective N_2-fixing association. Fall banding of the granular inoculant could ensure placement of the inoculant strain into moisture; therefore,

fall inoculation of first-time fields for the following spring planting should be investigated.

4. Under field conditions, soil inoculation enhanced seed protein concentration, even in situations where seed yield did not differ among inoculant treatments. This should be explored in relation to cooking and canning quality.

5. Laboratory and growth chamber conditions often do not reflect actual field conditions; hence, field investigation is required to confirm the results of the laboratory and growth chamber studies on fungicide-*Rhizobium* compatibility. The study should include the use of granular inoculant in combination with the fungicides tested.

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Appendix 1. Mean monthly precipitation and mean maximum temperature data for the experimental locations during the 1997 and 1998 growing seasons.

Month	Precipitation (mm)			Temperature (°C)		
	1997	1998	Normal	1997	1998	Normal
			Elbow			
May	26.4	36.2	49.0	17.2	20.4	18.1
June	101.8	78.8	53.3	23.0	20.5	23.0
July	18.4	6.0	56.9	26.6	27.2	26.1
August	46.8	22.4	35.8	26.4	28.9	25.6
			Outlook			
May	18.4	13.0	30.2	17.6	21.5	18.7
June	80.0	111.6	60.4	23.0	21.2	23.3
July	6.2	21.0	54.5	26.2	27.0	25.8
August	35.4	73.4	34.2	26.5	27.8	25.1
			Watrous			
May	65.2	54.8	51.2	17.1	20.0	18.1
June	106.4	147.4	69.1	23.4	19.9	22.7
July	17.6	34.6	59.0	25.4	25.0	25.3
August	64.0	39.6	37.6	25.8	27.5	24.6
			Davidson[†]			
May	47.0	61.5	40.6	16.7	19.9	18.1
June	70.3	159.1	58.3	23.2	19.5	22.7
July	18.6	68.2	55.8	25.8	25.0	25.8
August	54.9	40.7	38.7	25.6	27.3	25.1

Source: Environment Canada, Saskatoon, SK

[†]Data for Kenaston were not available, hence, data for Davidson (nearest station) are presented.

Appendix 2. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage, at Outlook, 1997.

Inoculant†	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g plant^{-1})
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoculated	0	0	0	0	0	0	1.23
Liquid A	0.60	4.80	5.40	12.0	11.5	23.5	1.24
Liquid C	0.45	5.25	5.70	1.5	8.5	10.0	1.07
Peat A	1.50	4.40	5.90	11.0	9.0	20.0	1.25
Peat B	3.00	6.80	9.80	18.5	19.0	37.5	1.04
Gran A ws	1.70	1.70	3.40	12.0	10.0	22.0	1.35
Gran A 2.5 cm	0.15	4.60	4.75	4.5	34.0	38.5	1.40
Gran A 8.0 cm	0.70	3.50	4.20	11.0	27.0	38.0	1.15
Gran B ws	2.25	8.35	10.60	14.0	26.5	40.5	1.05
Gran B 2.5 cm	0.35	4.05	4.40	3.5	38.5	41.5	1.40
Gran B 8.0 cm	0.45	4.35	4.80	8.5	41.5	50.0	1.49
$LSD_{(0.05)}$	1.55	5.24	5.66	11.1	18.9	25.3	0.33

† Gran = granular, ws = with seed.

Appendix 3. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Outlook, 1997.

Inoculant†	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g plant^{-1})
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoculated	0	0	0	0	0	0	3.70
Liquid A	1.05	0.85	1.90	26.0	31.0	57.0	4.14
Liquid C	2.10	1.90	4.00	37.0	24.0	61.0	4.34
Peat A	4.55	2.85	7.40	88.5	52.5	141.0	4.87
Peat B	2.65	1.80	4.45	72.5	19.5	92.0	4.78
Gran A ws	1.30	2.40	3.70	46.5	45.5	92.0	4.92
Gran A 2.5 cm	0.65	1.80	2.45	44.5	88.0	132.5	5.66
Gran A 8.0 cm	0	2.65	2.65	0	125.5	125.5	4.90
Gran B ws	1.95	2.65	4.60	68.0	47.5	115.5	4.54
Gran B 2.5 cm	1.55	2.15	3.70	79.5	90.0	169.5	5.36
Gran B 8.0 cm	0.60	3.25	3.85	16.5	108.0	124.5	6.05
$LSD_{(0.05)}$	1.56	1.22	2.47	65.7	55.0	84.7	1.44

† Gran = granular, ws = with seed.

Appendix 4. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Watrous, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.55	1.45	2.00	12.0	30.0	42.0	4.74
Liquid A	5.80	3.90	9.70	106.0	46.5	152.5	5.74
Liquid C	7.10	1.75	8.85	144.0	29.5	173.5	5.25
Peat A	4.75	2.30	7.05	102.5	60.5	163.0	5.02
Peat B	6.00	2.25	8.25	135.5	26.5	162.0	4.59
Gran A ws	4.00	4.05	8.05	114.0	73.0	187.0	6.33
Gran A 2.5 cm	0.75	3.75	4.50	7.5	150.0	157.5	6.66
Gran A 8.0 cm	1.25	3.95	5.20	22.0	110.0	132.0	5.86
Gran B ws	3.55	6.85	10.40	161.0	96.0	257.0	7.02
Gran B 2.5 cm	0.55	4.00	4.55	19.5	243.5	263.0	6.25
Gran B 8.0 cm	1.40	4.00	5.40	85.0	127.5	212.5	6.33
$LSD_{(0.05)}$	2.14	2.46	3.02	89.4	58.9	88.7	1.83

[†] Gran = granular, ws = with seed.

Appendix 5. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stage at Watrous, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.20	0.75	0.95	4.0	16.0	20.0	10.64
Liquid A	3.15	3.40	6.55	70.0	56.5	126.5	12.93
Liquid C	2.90	2.00	4.90	51.0	47.5	98.5	9.00
Peat A	7.05	5.50	12.55	171.0	105.5	276.5	14.16
Peat B	5.80	4.40	10.20	185.5	67.5	253.0	14.31
Gran A ws	3.25	6.30	9.55	68.0	82.5	150.5	13.65
Gran A 2.5 cm	0.80	4.90	5.70	44.0	147.0	191.0	17.09
Gran A 8.0 cm	0.25	4.40	4.65	2.0	170.5	172.5	15.24
Gran B ws	2.30	3.50	5.80	55.0	67.5	122.5	10.24
Gran B 2.5 cm	0.25	3.80	4.05	14.0	178.0	192.0	14.27
Gran B 8.0 cm	0.25	2.55	2.80	19.0	120.5	139.5	14.64
$LSD_{(0.05)}$	1.68	2.11	3.20	60.3	67.6	88.0	3.56

[†] Gran = granular, ws = with seed

Appendix 6. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage at Kenaston, 1997.

	Nodule no. plant^{-1}			Nodule dry wt (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.10	3.60	3.70	2.0	8.5	10.5	1.39
Liquid A	1.05	2.45	3.50	12.0	15.0	27.0	1.39
Liquid C	1.30	2.30	3.60	16.5	16.5	33.0	1.31
Peat A	2.50	3.80	6.30	53.0	42.0	95.0	1.47
Peat B	4.80	2.00	6.80	81.0	16.5	97.5	1.61
Gran A ws	1.30	4.15	5.45	29.5	67.5	97.0	1.82
Gran A 2.5 cm	0.50	4.35	4.85	6.0	70.0	76.0	2.03
Gran A 8.0 cm	0.40	2.45	2.85	8.0	75.5	83.5	1.87
Gran B ws	1.85	4.95	6.80	24.5	41.5	66.0	1.54
Gran B 2.5 cm	0.65	3.85	4.50	21.5	67.5	89.0	1.61
Gran B 8.0 cm	0.35	2.25	2.60	9.0	42.0	51.0	1.90
$LSD_{(0.05)}$	1.30	2.94	3.19	28.3	40.6	41.5	0.41

[†] Gran = granular, ws = with seed.

Appendix 7. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Kenaston, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.20	0.35	0.55	10.0	26.0	36.0	2.96
Liquid A	1.95	1.00	2.95	39.5	14.0	53.5	2.52
Liquid C	2.55	1.15	3.70	75.5	25.0	100.5	2.63
Peat A	3.65	1.20	4.85	120.5	38.5	159.0	3.69
Peat B	5.10	2.05	7.15	112.5	39.0	151.5	3.17
Gran A ws	1.95	1.50	3.45	53.0	45.0	98.0	2.51
Gran A 2.5 cm	0.40	2.05	2.45	15.0	98.5	113.5	3.79
Gran A 8.0 cm	0.25	1.00	1.25	2.5	29.5	32.0	3.65
Gran B ws	2.60	2.75	5.35	51.0	56.0	107.0	3.74
Gran B 2.5 cm	0.30	2.50	2.80	5.0	105.5	110.5	3.52
Gran B 8.0 cm	0.15	1.75	1.90	11.5	106.5	118.0	4.73
$LSD_{(0.05)}$	1.40	1.41	2.05	45.2	60.2	72.3	0.83

[†] Gran = granular, ws = with seed.

Appendix 8. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage at Watrous, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.45	0.65	1.10	6.0	1.5	7.5	1.43
Liquid A	3.60	2.20	5.80	47.0	13.0	60.0	1.70
Liquid C	2.45	4.30	6.75	68.5	16.0	84.5	1.34
Peat A	2.35	2.95	5.30	44.5	22.0	66.5	1.28
Peat B	2.65	3.45	6.10	54.5	20.0	74.5	1.63
Gran A ws	1.85	2.25	4.10	34.0	27.0	61.0	1.71
Gran A 2.5 cm	0.55	3.35	3.90	21.0	50.0	71.0	1.33
Gran A 8.0 cm	0.25	2.00	2.25	9.0	30.0	39.0	1.35
Gran B ws	0.90	2.75	3.65	19.0	35.0	54.0	1.46
Gran B 2.5 cm	0.65	3.15	3.80	26.5	58.0	84.5	1.45
Gran B 8.0 cm	0.35	2.30	2.65	5.5	25.5	31.0	1.23
$LSD_{(0.05)}$	1.46	1.86	2.38	41.3	28.3	48.2	0.40

† Gran = granular, ws = with seed.

Appendix 9. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the flowering stage at Elbow, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.05	0.10	0.15	1.0	1.5	2.5	0.88
Liquid A	0.25	0.25	0.50	2.5	1.0	3.5	0.81
Liquid C	0.50	1.25	1.75	5.0	5.5	10.5	1.04
Peat A	1.05	1.20	2.25	26.5	8.5	35.0	0.87
Peat B	1.65	1.05	2.70	21.5	8.0	29.5	0.88
Gran A ws	0.55	0.75	1.30	10.0	3.5	13.5	0.84
Gran A 2.5 cm	0.40	1.00	1.40	11.5	15.0	26.5	0.87
Gran A 8.0 cm	0	0.50	0.50	0	13.5	13.5	0.93
Gran B ws	1.25	2.00	3.25	16.0	13.0	29.0	0.89
Gran B 2.5 cm	0.65	1.65	2.30	21.0	24.5	45.5	0.97
Gran B 8.0 cm	0.30	1.30	1.60	3.5	8.0	11.5	0.97
$LSD_{(0.05)}$	0.84	1.20	1.52	16.4	11.3	22.2	ns

† Gran = granular, ws = with seed.

Appendix 10. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Elbow, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.15	0.40	0.55	4.5	14.5	19.0	3.45
Liquid A	0.30	0.30	0.60	19.5	15.0	34.5	3.08
Liquid C	0.45	0.65	1.10	6.0	9.0	15.0	3.88
Peat A	2.10	0.70	2.80	40.5	18.0	58.5	3.00
Peat B	3.50	1.15	4.65	80.0	35.0	115.0	3.45
Gran A ws	0.75	1.00	1.75	10.0	42.0	52.0	3.68
Gran A 2.5 cm	0.55	1.40	1.95	22.5	61.5	84.0	3.44
Gran A 8.0 cm	0	0.70	0.70	0	48.5	48.5	3.74
Gran B ws	1.80	1.20	3.00	50.0	21.0	71.0	3.59
Gran B 2.5 cm	0.70	1.05	1.75	16.0	44.0	60.0	3.58
Gran B 8.0 cm	0.30	1.40	1.70	5.5	77.5	83.0	4.02
$LSD_{(0.05)}$	0.95	0.73	1.26	20.8	38.0	6.7	ns

† Gran = granular, ws = with seed.

Appendix 11. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Watrous, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.50	0.45	0.95	5.5	18.0	23.5	7.74
Liquid A	2.80	2.30	5.10	70.5	42.0	112.5	7.62
Liquid C	5.20	2.50	7.70	103.5	34.0	137.5	7.25
Peat A	7.00	3.65	10.65	138.5	53.0	191.5	7.26
Peat B	7.55	5.20	12.75	180.0	79.5	259.5	9.51
Gran A ws	2.65	7.75	10.40	48.5	119.5	168.5	7.71
Gran A 2.5 cm	0.40	4.20	4.60	32.0	164.0	196.0	8.94
Gran A 8.0 cm	0.30	6.05	6.35	2.0	128.5	130.5	8.94
Gran B ws	2.80	6.30	9.10	48.5	95.0	143.5	8.61
Gran B 2.5 cm	0.95	4.15	5.10	35.5	165.0	200.5	9.56
Gran B 8.0 cm	0.75	3.35	4.10	29.0	143.5	172.5	9.16
$LSD_{(0.05)}$	2.65	3.09	3.98	88.5	62.9	105.6	ns

† Gran = granular, ws = with seed.

Appendix 12. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Kenaston, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.40	0.40	0.80	13.5	8.0	21.5	4.63
Liquid A	2.40	1.70	4.10	58.0	74.0	132.0	4.87
Liquid C	2.60	1.45	4.05	57.0	73.0	130.0	5.03
Peat A	4.45	2.40	6.85	219.0	81.0	300.0	5.84
Peat B	7.10	3.20	10.30	262.5	96.5	359.0	6.76
Gran A ws	3.20	3.30	6.50	115.0	113.0	228.0	6.93
Gran A 2.5 cm	0.60	6.75	7.35	8.5	184.5	193.0	8.95
Gran A 8.0 cm	0.50	4.60	5.10	6.5	117.0	123.5	7.55
Gran B ws	1.70	4.80	6.50	27.5	151.0	178.5	6.77
Gran B 2.5 cm	0.55	4.55	5.10	9.5	160.0	169.5	8.09
Gran B 8.0 cm	0.10	6.05	6.15	27.5	226.0	253.5	8.11
$LSD_{(0.05)}$	1.99	2.51	3.61	83.3	97.2	139.0	1.97

† Gran = granular, ws = with seed.

Appendix 13. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stage at Kenaston, 1997.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant†	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.75	0.25	1.00	32.0	50.0	82.0	6.91
Liquid A	1.35	1.75	3.10	65.5	97.5	163.0	7.66
Liquid C	4.25	2.45	6.70	158.5	199.5	358.0	9.32
Peat A	4.95	3.20	8.15	179.5	105.5	285.0	10.45
Peat B	4.80	6.50	11.30	184.0	205.5	389.5	10.77
Gran A ws	2.65	4.45	7.10	85.5	225.5	311.0	11.33
Gran A 2.5 cm	0.75	9.15	9.90	24.0	212.5	236.5	14.05
Gran A 8.0 cm	0.05	5.55	5.60	2.5	186.0	188.5	13.15
Gran B ws	1.55	8.00	9.55	30.0	222.5	252.5	14.00
Gran B 2.5 cm	0.70	5.70	6.40	25.5	230.5	256.0	12.65
Gran B 8.0 cm	0	5.50	5.50	0	219.0	219.0	16.05
$LSD_{(0.05)}$	1.12	1.90	2.28	73.1	103.6	138.2	3.02

† Gran = granular, ws = with seed.

Appendix 14. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the late pod-filling stage at Watrous, 1998.

Inoculant†	Nodule no.plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g plant^{-1})
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoculated	0	0.60	0.60	0	17.5	17.5	5.94
Liquid A	0.50	1.70	2.20	19.0	46.5	65.5	7.17
Liquid B	0.75	0.90	1.65	30.0	22.5	52.5	6.00
Peat A	2.50	1.75	4.25	68.0	40.0	108.0	8.75
Peat B	3.30	1.30	4.60	87.0	19.0	106.0	6.65
Gran A ws	3.00	4.15	7.15	77.5	84.5	162.0	11.09
Gran A 2.5 cm	0.90	6.30	7.20	14.5	111.5	126.0	11.33
Gran A 8.0 cm	0	5.60	5.60	0	92.0	92.0	8.45
Gran B ws	2.15	3.70	5.85	30.5	70.5	101.0	7.65
Gran B 2.5 cm	1.00	4.50	5.50	20.5	81.5	102.0	8.56
Gran B 8.0 cm	0.65	6.10	6.75	7.5	108.5	116.0	9.39
$LSD_{(0.05)}$	1.05	1.58	2.12	28.6	41.7	52.1	2.04

† Gran = granular, ws = with seed.

Appendix 15. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the late pod-filling stage at Watrous, 1998.

Inoculant†	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g plant^{-1})
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoculated	0	2.45	2.45	0	76.0	76.0	10.35
Liquid A	3.30	1.65	4.95	29.0	44.0	73.0	9.83
Liquid B	2.00	3.20	5.20	79.0	89.0	168.0	11.73
Peat A	3.60	1.65	5.25	151.5	52.5	204.0	12.82
Peat B	6.25	3.40	9.65	126.5	68.5	195.0	12.44
Gran A ws	2.80	5.00	7.80	69.5	101.0	170.5	13.99
Gran A 2.5 cm	0.85	7.05	7.90	11.5	138.5	150.0	13.66
Gran A 8.0 cm	0.35	10.00	10.35	14.5	195.5	210.0	14.72
Gran B ws	3.50	7.35	10.85	88.0	157.5	245.5	14.27
Gran B 2.5 cm	1.85	8.05	9.90	34.5	179.0	213.5	13.56
Gran B 8.0 cm	0.25	8.25	8.50	11.0	142.0	153.0	12.75
$LSD_{(0.05)}$	2.90	2.51	3.51	45.9	57.8	77.1	2.96

† Gran = granular, ws = with seed.

Appendix 16. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Outlook, 1998.

Inoculant†	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g plant^{-1})
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoculated	0	0.10	0.10	0	3.5	3.5	4.15
Liquid A	0.05	0	0.05	4.0	0	4.0	3.75
Liquid B	2.40	1.35	3.75	88.5	50.0	138.5	4.45
Peat A	4.15	2.75	6.90	217.5	90.0	307.5	4.30
Peat B	4.40	3.35	7.75	130.5	45.5	176.0	4.55
Gran A ws	2.00	6.45	8.45	60.0	169.5	229.5	5.05
Gran A 2.5 cm	0.20	6.20	6.40	3.5	200.5	204.0	5.85
Gran A 8.0 cm	0	5.75	5.75	0	133.0	133.0	6.40
Gran B ws	1.65	4.85	6.50	71.5	113.5	185.0	5.20
Gran B 2.5 cm	0.60	5.45	6.05	13.0	105.5	118.5	5.50
Gran B 8.0 cm	0	4.80	4.80	0	144.0	144.0	5.75
$LSD_{(0.05)}$	1.11	2.65	2.86	67.9	70.4	89.6	1.46

† Gran = granular, ws = with seed.

Appendix 17. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Myles desi chickpea at the early pod-filling stage at Watrous, 1998.

Inoculant†	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt. (g plant^{-1})
	Crown	Lateral	Total	Crown	Lateral	Total	
Non-inoculated	0	0.15	0.15	0	2.5	2.5	5.24
Liquid A	0.30	0.70	1.00	15.0	32.0	47.0	4.57
Liquid B	1.60	1.45	3.05	64.0	46.0	110.0	5.01
Peat A	2.40	2.00	4.40	169.0	51.5	220.5	5.96
Peat B	3.35	1.25	4.60	170.5	36.5	207.0	6.22
Gran A ws	2.85	4.45	7.30	100.5	156.5	257.0	7.47
Gran A 2.5 cm	2.05	6.25	8.30	40.0	164.0	204.0	6.04
Gran A 8.0 cm	0.80	7.65	8.45	15.5	259.5	275.0	7.66
Gran B ws	2.60	4.55	7.15	103.0	155.5	258.5	5.99
Gran B 2.5 cm	1.55	6.75	8.30	38.0	157.5	195.5	6.32
Gran B 8.0 cm	1.20	7.65	6.30	38.0	125.0	163.0	6.91
$LSD_{(0.05)}$	1.40	2.46	2.97	76.3	76.2	96.3	2.57

† Gran = granular, ws = with seed.

Appendix 18. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Watrous, 1998.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0.10	0.85	0.95	3.0	30.0	33.0	8.06
Liquid A	0.70	2.15	2.85	38.5	99.0	137.5	9.06
Liquid B	3.35	3.90	7.25	100.5	107.5	208.0	7.72
Peat A	3.65	3.00	6.65	157.5	105.0	262.5	10.65
Peat B	6.00	3.50	9.50	226.5	91.0	317.5	11.27
Gran A ws	3.95	6.65	10.60	91.5	128.0	219.5	9.28
Gran A 2.5 cm	1.60	9.75	11.35	21.0	175.5	196.5	10.30
Gran A 8.0 cm	1.15	8.60	9.75	17.5	183.5	201.0	10.74
Gran B ws	4.30	5.90	10.20	125.0	188.0	313.0	10.15
Gran B 2.5 cm	1.55	7.20	8.75	43.5	185.5	229.0	10.53
Gran B 8.0 cm	0.40	9.05	9.45	20.5	221.5	242.0	11.51
$LSD_{(0.05)}$	1.10	3.28	3.57	49.9	69.8	82.2	2.22

[†] Gran = granular, ws = with seed.

Appendix 19. Number of nodules, dry weight of nodules and dry matter production from various inoculation treatments of Sanford kabuli chickpea at the early pod-filling stage at Outlook, 1998.

	Nodule no. plant^{-1}			Nodule dry wt. (mg plant^{-1})			Shoot dry wt.
Inoculant[†]	Crown	Lateral	Total	Crown	Lateral	Total	(g plant^{-1})
Non-inoculated	0	0.15	0.15	0	13.0	13.0	5.01
Liquid A	0.40	0.10	0.50	14.5	2.5	17.0	6.50
Liquid B	1.55	1.55	3.10	38.0	38.5	76.5	5.06
Peat A	2.85	2.35	5.20	130.5	75.5	206.0	6.13
Peat B	3.75	3.00	6.75	103.5	67.5	171.0	7.33
Gran A ws	1.05	3.45	4.50	26.5	102.0	128.5	6.45
Gran A 2.5 cm	0.15	7.00	7.15	11.0	153.5	164.5	7.41
Gran A 8.0 cm	0	7.20	7.20	0	162.5	162.5	6.40
Gran B ws	1.70	4.30	6.00	59.5	113.5	173.0	7.80
Gran B 2.5 cm	1.25	4.80	6.05	27.0	81.5	108.5	7.51
Gran B 8.0 cm	0	3.95	3.95	0	53.0	53.0	5.11
$LSD_{(0.05)}$	0.92	2.67	2.73	47.9	46.4	62.5	2.42

[†] Gran = granular, ws = with seed.

Appendix 20. Whole plant bomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea at Elbow, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	1195	755	182	28.2	6.0
Liquid A	1215	705	176	29.1	5.9
Liquid C	1775	1030	196	47.0	15.7
Peat A	1643	960	202	48.8	13.8
Peat B	1500	965	181	57.9	18.7
Gran A with seed	1425	893	198	49.5	15.0
Gran A 2.5 cm bs	1430	880	223	51.6	17.8
Gran A 8.0 cm bs	1545	943	194	41.9	12.0
Gran B with seed	1258	738	191	51.1	12.0
Gran B 2.5 cm bs	1980	1173	226	65.5	28.6
Gran B 8.0 cm bs	1733	1043	188	61.1	18.7
$LSD_{(0.05)}$	678	355	33	16.5	10.8

† Gran = granular, bs = below seed

Appendix 21. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea at Outlook, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	1143	653	187	10.8	2.2
Liquid A	1628	875	199	32.8	9.1
Liquid C	1310	750	215	14.8	4.6
Peat A	1803	1100	213	44.9	16.8
Peat B	1260	773	216	48.1	13.6
Gran A with seed	1413	843	210	22.6	7.5
Gran A 2.5 cm bs	1755	1045	227	40.0	16.1
Gran A 8.0 cm bs	1610	923	224	48.6	17.4
Gran B with seed	1498	860	212	36.9	11.2
Gran B 2.5 cm bs	1498	888	216	38.7	12.1
Gran B 8.0 cm bs	1535	915	221	44.0	14.6
$LSD_{(0.05)}$	ns	373	22	17.6	8.6

† Gran = granular, bs = below seed.

Appendix 22. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea at Watrous, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	1823	820	172	23.8	6.0
Liquid A	2180	1053	193	36.9	12.9
Liquid C	2180	1095	185	51.0	17.6
Peat A	2698	1355	216	55.2	27.7
Peat B	1913	893	201	50.8	14.4
Gran A with seed	2230	1158	212	63.1	24.8
Gran A 2.5 cm bs	2598	1275	215	59.5	26.5
Gran A 8.0 cm bs	2808	1505	209	56.2	28.1
Gran B with seed	1993	985	206	46.3	15.1
Gran B 2.5 cm bs	2418	1170	208	52.8	20.7
Gran B 8.0 cm bs	2323	1125	199	54.2	19.3
$LSD_{(0.05)}$	591	316	17	13.3	8.8

† Gran = granular, ws = with seed.

Appendix 23. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea at Watrous, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	3200	1468	187	53.2	28.5
Liquid A	4118	1728	189	60.9	45.7
Liquid C	3820	1668	189	67.2	44.0
Peat A	4220	1790	204	74.2	58.2
Peat B	4305	1875	192	74.9	56.9
Gran A with seed	3805	1713	195	60.4	39.0
Gran A 2.5 cm bs	3955	1718	221	78.3	62.5
Gran A 8.0 cm bs	4443	1898	204	76.7	63.0
Gran B with seed	3278	1448	212	63.6	41.5
Gran B 2.5 cm bs	4113	1823	209	68.4	54.0
Gran B 8.0 cm bs	3828	1658	220	77.8	58.8
$LSD_{(0.05)}$	823	323	41	16.2	22.5

† Gran = granular, ws = with seed.

Appendix 24. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and the amount of seed N fixed for Sanford kabuli chickpea at Kenaston, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	1303	495	174	36.7	4.9
Liquid A	1440	590	184	52.9	9.2
Liquid C	1695	760	195	70.8	16.8
Peat A	2055	963	218	69.9	23.5
Peat B	2043	965	206	84.5	26.9
Gran. A with seed	1958	950	216	78.0	25.9
Gran A 2.5 cm bs	2843	1305	226	80.9	37.8
Gran A 8.0 cm bs	2363	1077	230	75.8	30.2
Gran B with seed	2143	965	222	83.4	28.9
Gran B 2.5 cm bs	2230	1010	215	85.8	30.1
Gran B 8.0 cm bs	2330	1085	218	79.7	30.3
$LSD_{(0.05)}$	497	282	17	10.1	8.9

† Gran = granular, ws = with seed.

Appendix 25. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea at Kenaston, 1997.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	1490	708	147	38.8	6.5
Liquid A	1778	875	182	43.0	10.7
Liquid C	1845	940	167	64.2	16.2
Peat A	2070	1070	192	66.7	22.2
Peat B	2245	1150	193	70.5	24.5
Gran A with seed	2155	1120	183	70.5	23.5
Gran A 2.5 cm bs	2608	1378	188	72.5	30.6
Gran A 8.0 cm bs	2713	1453	183	74.5	30.9
Gran B with seed	2418	1258	183	73.2	27.5
Gran B 2.5 cm bs	2288	1218	179	77.5	26.6
Gran B 8.0 cm bs	3127	1678	191	73.3	38.2
$LSD_{(0.05)}$	388	216	45	14.7	11.8

† Gran = granular, ws = with seed.

Appendix 26. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea at Outlook, 1998.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	2220	909	179	4.0	1.0
Liquid A	2340	943	178	6.7	1.7
Liquid B	2156	929	177	18.6	5.1
Peat A	2173	901	240	39.4	13.8
Peat B	2332	1075	218	40.7	15.3
Gran A with seed	2289	923	235	41.1	14.2
Gran A 2.5 cm bs	2705	911	249	42.1	15.6
Gran A 8.0 cm bs	2302	754	250	36.9	10.9
Gran B with seed	2476	1005	224	43.5	16.0
Gran B 2.5 cm bs	2054	754	237	36.9	10.4
Gran B 8.0 cm bs	1657	357	268	19.2	2.9
$LSD_{(0.05)}$	478	224	22	8.7	4.8

† Gran = granular, bs = below seed.

Appendix 27. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Sanford kabuli chickpea at Watrous, 1998.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	5265	1528	201	11.1	5.5
Liquid A	5128	1560	237	35.4	20.9
Liquid B	5278	1563	237	38.4	23.0
Peat A	5708	1733	237	41.7	27.0
Peat B	5585	1711	247	42.7	28.7
Gran A with seed	6433	1800	251	40.2	29.1
Gran A 2.5 cm bs	5675	1624	243	46.3	29.5
Gran A 8.0 cm bs	5823	1654	237	37.7	23.3
Gran B with seed	5648	1715	236	44.7	29.0
Gran B 2.5 cm bs	5685	1617	248	41.9	27.0
Gran B 8.0 cm bs	5318	1552	248	41.5	25.1
$LSD_{(0.05)}$	1079	ns	ns	10.5	7.7

† Gran = granular, bs = below seed.

Appendix 28. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea at Outlook, 1998.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	1486	846	143	6.6	1.4
Liquid A	2118	1156	153	11.8	3.3
Liquid B	2037	1152	145	36.3	10.4
Peat A	2639	1478	167	51.9	20.6
Peat B	2512	1455	184	49.2	22.2
Gran A with seed	2603	1462	185	55.3	24.4
Gran A 2.5 cm bs	2733	1504	229	62.8	34.4
Gran A 8.0 cm bs	2295	1310	234	57.9	28.3
Gran B with seed	2453	1411	193	46.0	19.9
Gran B 2.5 cm bs	2349	1412	209	54.9	26.4
Gran B 8.0 cm bs	2065	1210	203	50.9	28.3
$LSD_{(0.05)}$	551	318	26	14.2	8.9

† Gran = granular, bs = below seed.

Appendix 29. Whole plant biomass, seed yield, seed protein concentration, percentage N derived from atmosphere for the seed (%Ndfa) and amount of seed N fixed for Myles desi chickpea at Watrous, 1998.

Inoculant†	Biomass (kg ha^{-1})	Seed yield (kg ha^{-1})	Protein conc. (g·kg^{-1})	%Ndfa	N_2 fixed (kg ha^{-1})
Non-inoculated	3204	1598	170	27.7	12.1
Liquid A	3993	1886	184	27.9	16.3
Liquid B	3906	1839	202	51.7	31.5
Peat A	4369	2147	219	46.4	34.5
Peat B	4615	2048	249	58.1	47.5
Gran A with seed	4863	2148	215	58.4	43.7
Gran A 2.5 cm bs	4410	1993	245	57.8	44.8
Gran A 8.0 cm bs	4557	2070	226	60.6	45.4
Gran B with seed	4552	2099	222	63.8	47.5
Gran B 2.5 cm bs	4569	2051	234	54.3	41.7
Gran B 8.0 cm bs	4770	2242	250	56.1	50.2
$LSD_{(0.05)}$	575	282	25	12.0	10.9

† Gran = granular, bs = below seed.

Appendix 30. Nodule numbers of desi chickpea from various inoculation treatments at different growth stages for Experiment 1.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	Number of nodules plant^{-1}					
	Crown nodules					
Peat	0.25	1.75	2.63	3.13	2.25	1.63
Liquid	0	0	0	0	0	0
Granular	0	0	0	0	0	0
Non-inoculation	0	0	0	0	0	0
$LSD_{(0.05)}$	ns	0.70	1.58	2.00	1.65	0.89
	Lateral root nodules					
Peat	0	0.50	0.25	0.63	0.63	0.13
Liquid	0	0	0	2.13	1.38	0.13
Granular	0.50	2.25	3.25	0.25	3.13	3.00
Non-inoculation	0	0.13	3.00	0.13	0	0
$LSD_{(0.05)}$	0.46	0.98	ns	1.75	2.53	0.71
	Total					
Peat	0.25	2.25	2.85	3.75	2.88	1.75
Liquid	0	0	0	0.25	1.38	0.13
Granular	0.50	2.25	3.25	2.13	3.13	3.00
Non-inoculation	0	0.13	3.00	0.13	0	0.25
$LSD_{(0.05)}$	ns	1.63	ns	2.74	2.72	0.93

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Appendix 31. Nodule numbers of desi chickpea from various inoculation treatments at different growth stages for Experiment 2.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	Number of nodules plant^{-1}					
	Crown nodules					
Peat	2.63	3.13	4.38	4.38	2.88	3.75
Liquid	0.50	0.13	0.38	0.38	0.50	0
Granular	0	0	0	0	0	0
Non-inoculation	0	0	0	0	0	0
$LSD_{(0.05)}$	0.71	1.09	2.05	2.16	1.17	1.25
	Lateral root nodule					
Peat	1.13	1.63	2.25	0.63	0.88	0.38
Liquid	0.50	0.50	0.75	0.63	0.13	0.75
Granular	1.63	3.75	4.00	4.25	3.75	4.63
Non-inoculation	0	0	0.25	0.75	0.38	0
$LSD_{(0.05)}$	0.97	1.52	2.05	2.15	1.31	1.32
	Total					
Peat	3.75	4.75	6.63	5.00	3.75	4.13
Liquid	1.00	0.63	1.13	1.00	0.63	0.75
Granular	1.63	3.75	4.00	4.25	3.75	4.63
Non-inoculation	0	0	0.25	0.75	0.38	0
$LSD_{(0.05)}$	0.85	1.76	3.49	2.57	1.46	1.89

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Appendix 32. Nodule dry weight of desi chickpea from various inoculation treatments at different growth stages for Experiment 1.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	Crown nodules (mg plant^{-1})					
Peat	0.8	41.3	62.1	84.2	78.3	86.1
Liquid	0	0	0	0	0	0
Granular	0	0	0	0	0	0
Non-inoculation	0	0	0	0	0	0
$LSD_{(0.05)}$	ns	15.7	60.6	54.1	52.3	58.2
	Lateral root nodules (mg plant^{-1})					
Peat	0	15.9	34.9	18.6	92.0	45.0
Liquid	0	0	0	14.7	52.2	33.0
Granular	10.2	50.7	162.5	108.5	218.3	210.5
Non-inoculation	0	2.9	62.0	1.5	0	63.2
$LSD_{(0.05)}$	ns	27.6	95.2	84.1	200.5	139.8
	Total nodule dry wt (mg plant^{-1})					
Peat	0.8	57.2	97.0	102.3	170.8	131.2
Liquid	0	0	0	14.7	52.2	33.0
Granular	10.2	50.7	162.5	108.5	231.8	210.5
Non-inoculation	0	2.9	62.0	1.5	0	63.2
$LSD_{(0.05)}$	ns	41.4	96.2	85.8	197.5	143.1

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Appendix 33. Nodule dry weight of desi chickpea from various inoculation treatments at different growth stages for Experiment 2.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	Crown nodules (mg plant^{-1})					
Peat	42.0	75.3	112.2	105.2	94.9	122.0
Liquid	15.8	4.5	29.4	30.3	7.9	0
Granular	0	0	0	0	0	0
Non-inoculation	0	0	0	0	0	0
$LSD_{(0.05)}$	24.7	21.6	65.6	74.1	23.2	28.1
	Lateral root nodules (mg plant^{-1})					
Peat	8.3	49.2	59.4	18.5	42.7	14.3
Liquid	3.4	28.3	38.9	27.3	125.0	24.5
Granular	39.5	136.0	140.2	138.3	183.3	155.8
Non-inoculation	0	0	23.2	30.2	35.5	0
$LSD_{(0.05)}$	20.4	55.2	74.2	78.2	ns	52.9
	Total nodule dry wt (mg plant^{-1})					
Peat	50 .3	124.4	171.5	168.9	137.5	136.3
Liquid	19.2	32.3	68.3	57.5	132.9	24.5
Granular	39.5	136.0	140.2	138.3	183.3	155.8
Non-inoculation	0	0	23.2	30.2	35.5	0
$LSD_{(0.05)}$	42.3	53.5	48.3	90.1	ns	50.6

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

Appendix 34. Dry matter production of desi chickpea from various inoculation treatments at different growth stages for Experiment 1.

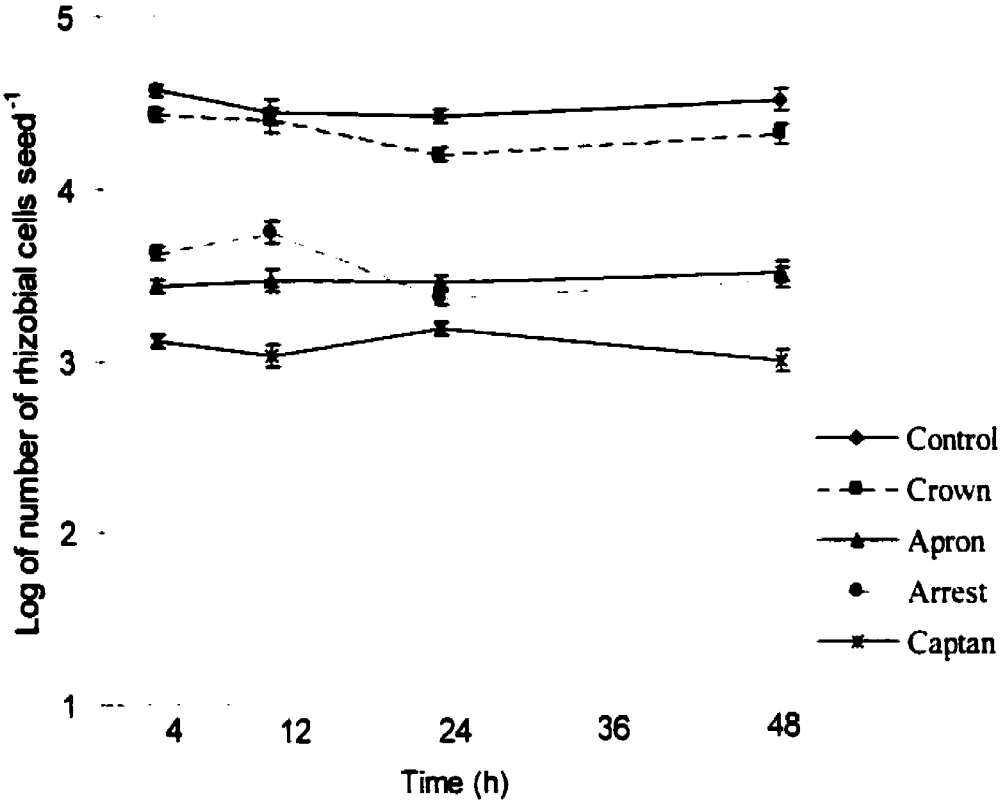
Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	g plant^{-1}					
Peat	0.95	2.16	3.22	4.26	4.60	4.82
Liquid	0.88	2.03	3.09	4.00	4.14	3.88
Granular	1.04	2.26	3.57	4.07	5.08	6.03
Non-inoculation	0.93	2.21	2.97	3.77	3.52	4.84
$LSD_{(0.05)}$	ns	ns	ns	ns	0.72	1.03

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.

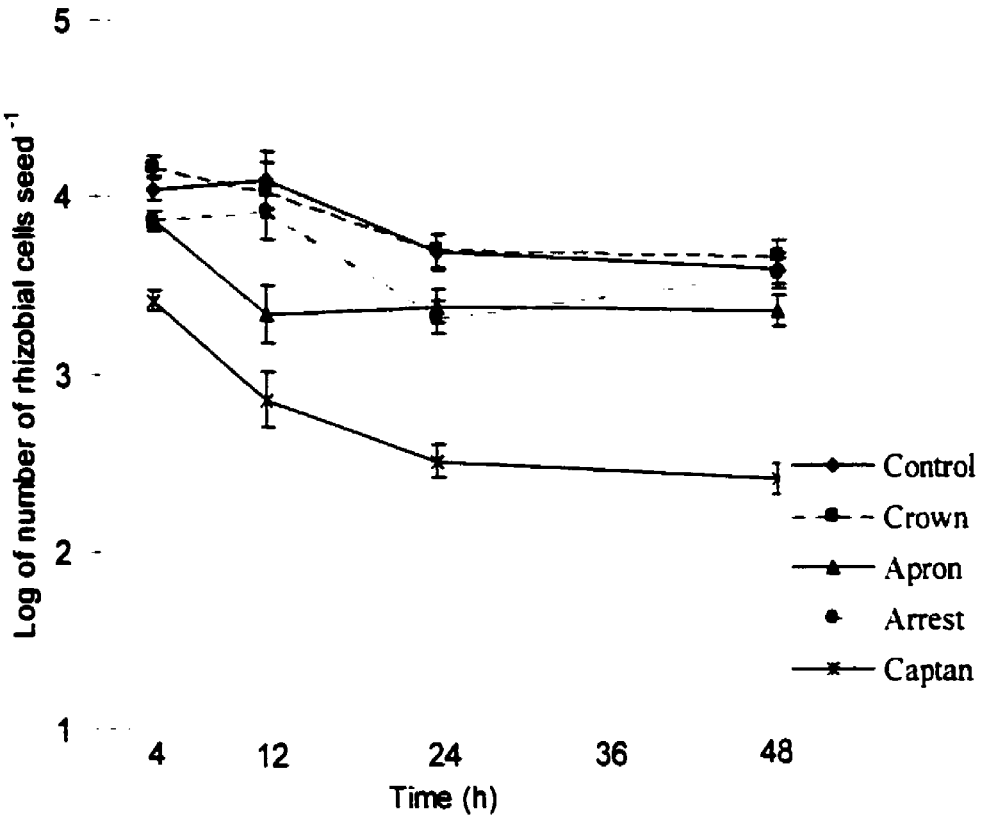
Appendix 35. Dry matter production of desi chickpea from various inoculation treatments at different growth stages for Experiment 2.

Inoculation treatment	Days after planting (DAP)†					
	28	42	56	70	84	98
	g plant^{-1}					
Peat	1.27	1.96	2.83	3.12	2.98	3.42
Liquid	1.11	1.71	2.00	2.45	2.04	2.54
Granular	1.23	1.99	2.78	3.03	3.23	3.59
Non-inoculation	1.14	1.70	2.11	2.05	1.90	1.88
$LSD_{(0.05)}$	ns	ns	0.48	0.75	0.59	0.74

† 28 DAP = late vegetative, 42 DAP = flowering, 56 DAP = early pod-filling, 70 DAP = mid pod-filling, 84 DAP = late pod-filling and 98 DAP = physiological maturity.



Appendix 36. Survival of *R. ciceri* strain CP39 on seeds treated separately with one of four fungicides seven days prior to inoculation as compared to the inoculated, but fungicide-free, control in Experiment 1. Each point is the mean of four replications, with vertical bars representing standard error.



Appendix 37. Survival of *R. ciceri* strain CP39 on seeds treated separately with one of four fungicides seven days prior to inoculation as compared to the inoculated. but fungicide-free, control in Experiment 2. Each point is the mean of four replications, with vertical bars representing standard error.