EFFECTS OF CO-INOCULATION WITH *BACILLUS CEREUS* UW85 AND (BRADY)RHIZOBIA ON THE NODULATION, NITROGEN FIXATION AND DRY MATTER ACCUMULATION OF GRAIN LEGUMES

BY
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A Thesis
Submitted to the Faculty of Graduate Studies
in Partial Fulfilment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Plant Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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Abstract

Effects of Co-inoculation with *Bacillus cereus* UW85 and (Brady)Rhizobia on the Nodulation, Nitrogen Fixation and Dry Matter Accumulation of Grain Legumes

Terry J. Buss, Department of Plant Science, University of Manitoba, Major Professor, Dr. J. Kevin Vessey.

Putative evidence, from both laboratory and field experiments by other researchers, has indicated that *Bacillus cereus* UW85 can increase soybean (*Glycine max* L. Merr) growth and yield by promoting nodulation and N_2 fixation. That work involved only soybeans in situations where indigenous *Bradyrhizobium japonicum* was often present. The objective of my research was to access the effects of co-inoculation with *B. cereus* UW85 and (Brady)Rhizobia on the nodulation, N_2 fixation, and DM accumulation of soybean (*Glycine Max* L. Merr.), field bean (*Phaseolus vulgaris* L.), field pea (*Pisum sativum* L.) and lentil (*Lens esculenta* Moench) both in the growth chamber and in the field.

In gnotobiotic growth chamber experiments, soybean inoculated with *B. cereus* UW85 and higher rates of *B. japonicum* (10^6 cell ml^{-1}) demonstrated greater plant part DM accumulations, N contents and root nodule numbers than soybeans inoculated with higher rates of *B. japonicum* alone. Soybean inoculated with *B. cereus* UW85 and lower rates of *B. japonicum* (10^2 cell ml^{-1}) had greater root DM than soybeans inoculated with lower rates of *B. japonicum* alone . No promotions of specific root nodulation or specific nitrogenase activity were found. Root N concentrations were "diluted" through *B. cereus* UW85 inoculation. Common bean seedlings were damaged and their emergence reduced by *B. cereus* UW85 inoculation. Field pea, tested in two temperature regimes,

demonstrated limited positive and negative responses to *B. cereus* UW85 inoculation.

In field experiments, the four legume species were inoculated with a granular formulation of *B. cereus* UW85. At Winnipeg, soybean demonstrated increased plant part DM accumulations, N concentrations, N contents and seed yield . Promotions occurred in the absence of root nodules and nitrogen fixation. No growth promotions occurred with soybean at Carman. The growth of common bean, field pea and lentil was not promoted by *B. cereus* UW85 in the field.

It is suggested that *B. cereus* UW85 promotes the growth of soybean roots by some unknown mechansim(s). Increases in root nodulation were a consequence of an increase in potential infection sites.

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1.0 Introduction

The term plant growth-promoting rhizobacteria (PGPR), was first used by Kloepper and Schroth (1978) to describe bacteria that, when applied to seeds, tubers or roots, caused a promotion of plant growth. Because of a shift in focus towards issues of pollution, food safety and the use of non-renewable resources in agriculture, PGPR have become an active area of research interest (Jacobson and Backman 1993; Glick 1995). In fact, PGPR products already exist in the marketplace (Okon 1985; Okon and Hadar 1987; Turner and Backman 1991; Mahafee and Backman 1993). PGPR are not a new idea and have had widespread use in the past. Brown (1974) has documented that "bacterization treatments" were used on over 35 million acres of crop land in areas belonging to the former Soviet Union in 1958.

Through the years, genera used both in research and in field-scale agriculture have included *Azotobacter, Azospirillum, Bacillus, Pseudomonas, Arthrobacter, Enterobacter, Serratia* and *Clostridium* (Burr and Caesar 1984; Kapulnik 1991; Glick 1995). The genera *Rhizobium* and *Bradyrhizobium* could be considered the most significant PGPR to date because of their ability to "fix" atmospheric N_2 while in a symbiotic relationship with host plants (Vance 1991; Glick 1995). However, these organisms have typically not been grouped with PGPR in the scientific literature. PGPR are most often considered to be exclusively free-living bacteria (Glick 1995; Kloepper et al. 1989). In contrast to PGPR, *Rhizobium* and *Bradyrhizobium* can exist within discrete plant root structures (nodules) and form an established symbiotic relationship with the plant (Vance 1991).

Many organisms considered to be PGPR came out of searches for bio-control

agents. According to Jacobsen and Backman (1993), the criteria that distinguishes between PGPR and bio-control agents is vague. This becomes evident when the proposed modes of action for PGPR are considered. Glick (1995) divided PGPR modes of action into two categories. Indirect mechanisms was the first category. These involve PGPR being able to reduce the deleterious effects of one or more phytopathogenic organisms through various mechanisms (i.e. siderophore production, antibiotic production, competition). Suslow and Schroth (1982) examined phytopathogenic organisms they labelled as deleterious rhizobacteria (DRB). They found the effects of such organisms on plant growth to be subtle and proposed that such organisms are ubiquitous and common to all root systems. In their view, at least part of the reason why the PGPR they examined promoted plant growth was that these PGPR inhibited the growth of pathogenic organisms not identified with any established disease. The second category, as outlined by Glick (1995), was direct mechanisms. Here, PGPR synthesize compounds in the rhizosphere which promote plant growth (i.e. phytohormones) or facilitate the uptake of certain nutrients by the plant. Glick (1995) noted that the area of direct mechanisms had received the least attention in the literature.

In this thesis, the potential of *Bacillus cereus* strain UW85 as a PGPR in a variety of legumes was examined in both growth chamber and field studies. The strain was used as a treatments on soybean (*Glycine max* L. Merr.), field bean (*Phaseolus vulgaris* L.), field pea (*Pisum sativum* L.) and lentil (*Lens esculenta* Moench). It was hypothesized that the strain would be found to promote the growth and seed yield of plants it was applied to in the growth chamber and in the field. Furthermore, it was expected that

application of the strain would result in increased plant tissue N content, nitrogenase activity and root nodule numbers in treated plants.

2.0 Literature Review

While studies involving PGPR have been conducted on many crop types, the relationships between PGPR and legumes are of particular relevance to this thesis. In the following review, experimental evidence of PGPR activity with legumes will be considered in the context of three categories of growth promotion. These include: 1) the promotion of seedling emergence and vigour, 2) the promotion of the (Brady)Rhizobia-legume symbiosis and 3) the promotion of seed yield, the growth of plant parts and the nutrient content of those plant parts.

2.1 Enhancement of Seedling Emergence and Vigour

When examining work that deals with PGPR promoting seedling emergence and vigour, one has to keep in mind that PGPR can also be bio-control organisms depending on the context. Seedling diseases caused by fungi belonging to the genera *Pythium, Rhizoctonia, Fusarium* and *Phytophthora* cause significant early season damage to many crops including legumes (Reddy et al. 1993; Howell et al. 1988; Lifshitz et al. 1986). Organisms that have been classified as PGPR or share the same genera as known PGPR have been found to act as bio-control agents against these fungi when used as inoculants with different plant species. Howell et al. (1988) were able to demonstrate that two *Enterobacter cloacae* strains showed progressive symmetrical inhibition to *Rhizoctonia solani* and *Pythium ultimum* which had been streaked on quad-partitioned plates. This occurred even though the organisms made no physical contact on the plates. It was suggested that ammonium production by *E. cloacae* was toxic to the fungi at low

concentrations.

Reddy et al. (1993) found 23 strains, all from the *Pseudomonas* genera, which suppressed either *P. ultimum, R. solani or Fusarium solani* f.sp. *phaseoli* on white bean seedlings (*Phaseolus vulgaris* L.) in greenhouse experiments using infected field soil. They determined that there was no relationship between in vitro antibiosis and a strain's effectiveness in greenhouse testing. Using several *Pseudomonas* strains isolated from common bean roots infected by *Pythium* sp., Elad and Chet (1987) were able to isolate six strains that yielded between 60 and 90% disease reduction in greenhouse experiments. They suggested that active rhizosphere colonization and niche exclusion of *Pythium aphanidermatum* by their selected strains resulted in the observed control. Lifshitz et al. (1986) found 13 rhizobacteria strains that provided control of soybean seedling wilt, lateral root stunting and seedling death caused by *Phytophthora megasperma* f.sp. *glycinea* in greenhouse studies. The effective strains were identified as *Pseudomonas putida* sp. and *Pseudomonas fluorescens* sp..

The work outlined above demonstrates that PGPR-like strains have been implicated in the control of identified parasitic fungi on legumes. There are cases in the literature where promotions of emergence, vigour and seedling survival are seen while no identified pathogens can be implicated. Kloepper et al. (1986) screened strains from the *Pseudomonas* genera for their ability to promote the emergence of soybean seedlings in cold soils. Experiments were conducted in a greenhouse at 14°C. Twenty three strains resulted in 50% greater emergence for treated plants as compared to controls. The authors noted that no distinct visual observations of disease were made in any of their

experiments.

There have been reports in the literature that some PGPR organisms produce siderophores which make iron unavailable in the rhizosphere for plant pathogens (Kloepper et al. 1980; Loper 1988). Suslow and Schroth (1982) have implicated deleterious rhizobacteria (DRB) as a cause of reduced seed germination. To determine if the strains in their experiments were producing siderophores that were inhibiting the action of DRB present in their planting medium, Kloepper et al. (1986) added 10^{-3} M $FeCl_3$ to soil being used in soybean emergence tests. The presence of an oversupply of available iron was expected to allow DRB to continue to grow and act in spite of siderophore production. All effective strains were found to retain their emergence-promoting abilities.

Kloepper et al. (1986) concluded that rhizobacteria which would reliably promote the emergence of soybean (*Glycine max* L. Merr.) offered up three advantages. First, with a shortened time to emergence, longer season and potentially higher yielding cultivars could be grown in shorter season areas. Second, the acceleration of seedling emergence due to bacterial treatments could reduce the incidence of seedling diseases. Third, in areas where prolonged dry periods tend to occur after seedling emergence, an acceleration of seedling growth would allow plants to establish more root mass to take advantage of moist soil conditions for as long as they exist.

In field studies, Chanway et al. (1989) found that inoculation of lentil cv. Eston with *P. putida* strain G11-32 and *Rhizobium leguminosarum* bv. *viceae* strain 175P1 resulted in 23% greater seedling emergence compared to lentil inoculated with *R.*

leguminosarum bv. *viceae* alone at 11 days after planting (DAP). A visual estimate of seedling vigour at 11 DAP revealed that inoculation with *P. putida* strain G14-32 and *R. leguminosarum* bv. *viceae* strain 175P1 had increased the assessed vigour of lentil seedlings compared to inoculation with *R. leguminosarum* bv. *viceae* alone. Since growth promotions were also observed in sterile controlled environments, the authors concluded that the PGPR strains were promoting growth directly by mechanisms other than the antibiosis of identified or unidentified pathogens.

Turner and Backman (1991) monitored the emergence and subjectively rated the vigour of peanut cultivars (*Arachis hypogaea* L.) that were treated with *Bacillus subtilis* strain A-13 as Quantum 4000 (Gustafson, Inc., Dallas, TX) In 1983 an increase in emergence was recorded in only one of 16 unreplicated field trials. In five replicated studies conducted from 1983 to 1985, only one 21% increase in emergence was found. This emergence promotion was correlated to low soil temperatures. In these experiments, visibly detectable differences in vigour between plants inoculated with the test strain and controls were noted. Because seed used in experiments was treated with Pro-Ized II fungicide consisting of 10% Botran (DCNA) and 16% Thiram (thiuram) by weight (Gustafson, Inc., Dallas, TX), the authors felt emergence promotions were due to some unidentified property of strain A-13 that was protecting peanut seeds from physiological stresses in cold soils.

Table 2.1 summarizes the PGPR-mediated seedling emergence promotions previously described. It is important to note that substantial increases have been demonstrated with a variety of crops using a variety of PGPR.

Table 2.1: Summary of PGPR-mediated seedling emergence responses (+/- % change) discussed in the literature review.

Crop	PGPR	Seedling Emergence (%)	Reference
Soybean	*Pseudomonas* sp.	+50	Kloepper et al. (1986)
Lentil	*P. putida*	+23	Chanway et al. (1989)
Peanut	*B. subtilis*	+21	Turner and Backman (1991)

2.2 Promotion of the (Brady)Rhizobia-Legume Symbiosis

Since this review focuses on PGPR as they relate to legumes, the potential for enhancing nodulation and N_2 fixation must be examined. Vance (1991) stated that, "Acquisition and assimilation of N is second in importance only to photosynthesis for plant growth and development." Vance (1991) has also noted that symbiotically fixed N is the primary source for crops and soils in the developing world because of the high costs and infrastructure requirements of synthetic fertilizer production. A trend towards increased concerns over the use of non-renewable resources in agriculture in the developed world has been noted. If PGPR were found that reliably enhanced symbiotic N_2 fixation, there would be significant worldwide implications for agriculture.

The enhancement of legume nodulation or N_2 fixation through the use of PGPR is an area that received attention very early in the history of PGPR research (Brown 1974). For example, Harris (1953) found that *Rhizobium leguminosarum* biovar *trifollii* strains incapable of nodulating subterranean clover (*Trifolium subterraneum* L.) in sterilized soils, were effective in unsterilized soils. He proposed that *R. leguminosarum* strains were benefiting from interaction with indigenous soil bacteria. More recent reviews of the subject by Kloepper et al. (1989), Kapulnik (1991) and Beauchamp (1993) have demonstrated that this interest continues. In a discussion of microbial interactions on roots, Bowen and Rovira (1991) stated that, "Theoretically, biological control of symbionts is just as real a possibility as biological control of pathogens or harmful soil microorganisms." Kloepper et al. (1989) have noted that bacterial stains which demonstrate long-term rhizosphere persistence and an ability to promote plant growth

could be used for the root-zone delivery of compounds that would enhance the activity of symbionts. The following review of the literature pertaining to the promotion of legume nodulation and N_2 fixation by PGPR will be organized along genera lines including *Azospirillum*, *Azotobacter*, *Pseudomonas*, and *Bacillus*.

Singh and Subba Rao (1979) conducted greenhouse experiments where soybean (cv. Clark-63) were grown in pots of unsterilized soil and inoculated with *Azospirillum brasilense* strains Madhu or Sp-7 with or without *Bradyrhizobium japonicum* strain SB-16. Inoculation with *A. brasilense* strain Madhu alone resulted in 9956% greater root nodule numbers and a 667% greater nodule dry weights compared to uninoculated controls. Soybean inoculated with both *A. brasilense* strain Madhu and *B. japonicum* strain SB-16 demonstrated 32 to 67% greater root nodule numbers than soybean inoculated with either strain alone. The authors concluded that *A. brasilense* strain Madhu was making nodulation by *B. japonicum* indigenous to the potting soil more successful.

Raverkar and Konde (1988) obtained similar results in a field experiment involving the inoculation of peanut (cv. Robut 33-1 and JL 24) with *Azospirillum lipoferum* strain ICM 1001 with or without *Rhizobium* sp. strain NC 92. Inoculation of Robut 33-1 peanut with the *A. lipoferum* strain alone resulted in 47% greater root nodule numbers and 70% greater nodule dry weights compared to that found for uninoculated control plants. A 31% increase in nodule dry weights was found for cultivar JL 24 peanut inoculated with the *A. lipoferum* strain alone compared to uninoculated controls although no differences in root nodule numbers were found. Promotions of nodulation and plant growth were attributed to the production of growth-promoting substances by the *A.*

lipoferum strain.

Sarig et al. (1986) conducted both greenhouse and field experiments where a mixture of *A. brasilense* strains Cd, Sp 7 and Cd-1 was applied to vetch (*Vicia sativa* L., cv. Asor), garden pea (*Pisum sativum* L., cv. Perfection), sulla clover (*Hedysarum coronarium* L.) and chickpea (*Cicer arietinum* L., cv. California). In the greenhouse, a 19% increase in root nodule numbers was found for garden pea. No increases in nitrogenase activity (ARA) were found. In the field, increases in nitrogenase activity (ARA) of 113% and 74% were found for vetch and sulla clover, respectively when they had been inoculated with the *A. brasilense* mixture compared to uninoculated controls. No increases in root nodule numbers were observed. It was hypothesized that the *A. brasilense* strain mixture improved plant nutrient uptake which resulted in increased root nodule numbers and elevated nitrogenase activity levels.

Burns et al. (1981) conducted several experiments involving soybean (*Glycine max* L. Merr., cv. Ransom), sweet clover (*Trifolium repens* L., cv. Tilman White Ladino) and cowpea (*Vigna unguiculata* L., cv. Blackeye #5) inoculated with five strains of *Azotobacter vinelandii* . Three of the strains were nif$^-$ mutants. Note that N_2 fixation is controlled by genes on plasmids within N_2 fixing bacteria. Their presence is required for the process of N_2 fixation to take place. Nif$^-$ mutants lack these genes and are unable to fix N (Vance 1991). In greenhouse pot experiments, inoculation of plants with any of the *A. vinelandii* strains and the appropriate *B. japonicum* sp. or *Rhizobium* sp. increased root nodule numbers from 20 to 465% compared to plants inoculated with the appropriate *B. japonicum* sp. or *Rhizobium* sp. alone. Individual nodules were found to be smaller and

no differences in nitrogenase activity (ARA) among treatments were noted. The nif status of strains played no role in the enhancements of nodulation observed leaving the authors to conclude that the ability of *A. vinelandii* to fix N played no role. Inoculation treatments consisting of autoclaved, streptomycin killed or cell-free preparations of the *A. vinelandii* strains used resulted in no stimulation of nodulation. Split-root inoculation experiments using the *A. vinelandii* strains demonstrated that no plant translocatable compound was involved in the enhancements seen. The authors suggested that a non-excretable protein, produced by the *A. vinelandii* strains was responsible for the observed effects.

Plazinski and Rolfe (1985a) investigated how five *Azospirillum* sp. strains, applied with nine *R. leguminosarum* bv. *trifollii* strains, influenced the nodulation of white clover (*Trifolium variegatum* L., cv. New Zealand white clover 5826) and subterranean clover (*Trifolium subterraneum* L.). Observations were made on seedlings grown on plates and in water suspensions. Seven *R. leguminosarum* biovar *trifollii* strains, when inoculated with any of the five *Azospirillum* sp. strains, produced increases in root nodule numbers that ranged from 25 to 100%. The extra nodules produced were found to be inactive when assayed (ARA). Using non-viable *Azospirillum* sp. cells in inoculant formulations or placing *Azospirillum* sp. in dialysis bags which were soaked with plants in liquid media resulted in no stimulations of nodulation. The authors suggested that the *Azospirillum* sp. strains may have produced an excretable compound which created new infection sites on the clover roots.

In a related study, Plazinski and Rolfe (1985b) found that, through the addition of

auxins (IAA or NAA) to white clover seedlings grown in plate culture, they could mimic some of the increases in nodulation that they had produced with various *Azospirillum* sp. and *R. leguminosarum* bv. *trifollii* strain combinations. They suggested that phytohormone production by the *Azospirillum* strains played a role in nodulation enhancements.

Yahalom et al. (1987) examined the effects on nodulation and N_2 fixation of inoculating Bur clover (*Medicago polymorpha* L.), Siratro (*Macroptilium atropurpureum* L.) and Berseem clover (*Trifolium alexandrinum* L., cv. Tabor) with *Azospirillum brasilense* strain Cd and the appropriate *Rhizobium* sp. strains. Plants were grown in growth pouches under sterile conditions. Bur clover plants that had been inoculated with *A. brasilense* strain Cd and *Rhizobium* sp. had 30% greater root nodule numbers than plants inoculated with the *Rhizobium* sp. alone. As well, this inoculation led to 1000% greater nitrogenase activity (ARA) at seven days after inoculation and 392% greater nitrogenase activity at 9 days after inoculation compared plants inoculated with the *Rhizobium* sp. alone. Siratro inoculated with *A. brasilense* strain Cd and *Rhizobium* sp. demonstrated 25% greater nitrogenase activity at 13 days after inoculation and 80% greater nitrogenase acitivity at 20 days after inoculation compared to plants inoculated with the *Rhizobium* sp. alone. The authors suggested that *A. brasilense* strain Cd may have produced plant growth regulator compounds that stimulated root hair production which then either opened up new sites for nodule formation or increased plant nutrient uptake.

Using *Pseudomonas putida* strains M17 and M174, Grimes and Mount (1984)

investigated nodulation stimulation in common bean (*Phaseolus vulgaris* L., cv. Provider) in growth chamber and field studies. In sterile potting mix, the inoculation of seeds with either of the *P. putida* strains and *R. leguminosarum* bv. *phaseoli* resulted in 85 to 92% greater root nodule numbers compared to plants inoculated with the *R. leguminosarum* bv. *phaseoli* strain alone. Using a field soil in a growth chamber experiment, inoculation with *P. putida* strain M174 led to a 70% increase in nodulation by indigenous rhizobia compared to uninoculated controls. Inoculation with either *P. putida* strain and *R. leguminosarum* bv. *phaseoli* led to 93 to 96% greater nodule numbers versus inoculation with *R. leguminosarum* bv. *phaseoli* alone. In the field over two growing seasons, inoculation with either *P. putida* strain alone produced 79 to 300% greater root nodule numbers compared to uninoculated controls. As well, plants inoculated with either *P. putida* strain and *R. leguminosarum* bv. *phaseoli* demonstrated 15 to 92% greater root nodule numbers compared to plants inoculated with *R. leguminosarum* bv. *phaseoli* alone. Grimes and Mount (1984) found that their *P. putida* strains produced 2-ketogluconic acid, an effective soil phosphate-solubilizing compound, and they proposed that improved P nutrition of common bean plants was responsible for their results. Mullen et al. (1988) have shown that adequate P nutrition is essential for optimum root nodule numbers in soybean plants.

Polonenko et al. (1987) tested 18 *Pseudomonas* sp. strains for their ability to enhance the nodulation and nodule mass of soybean (cv. Maple Arrow) inoculated with *B. japonicum* strains 110 or 118 in greenhouse experiments. In field soil that contained no indigenous *B. japonicum*, seven of the strains resulted in 42 to 91% more root nodules

and 11 of the strains resulted in 34 to 109% greater nodule masses compared to plants inoculated with either of the *B. japonicum* strains alone. More promotions were seen when *B. japonicum* strain 110 was used. In sterile soil-less mix, two of the strains resulted in 143 to 164% greater root nodule numbers and one of the strains resulted in 371% greater nodule masses compared to plants inoculated with *B. japonicum* strain 110 alone. None of strains produced root nodule number or nodule mass promotions when treatments included *B. japonicum* strain 118. Several reductions in nodule number and nodule mass were noted for both of the *B. japonicum* strains. The authors concluded that the effective strains had either stimulated the nodulation process or plant growth directly.

Alagawadi and Gaur (1988) investigated the effects of inoculating chickpea (cv. BG 209) with *Pseudomonas striatia* strain 27 or *Bacillus polymyxa* stain H5, in greenhouse experiments using unsterilized soil mixed with farm manure. At 90 DAP, treatments including inoculation with *P. striatia* or *B. polymyxa* and *Rhizobium* sp. resulted in 41 to 50% greater root nodule numbers than the treatment which included only the *Rhizobium* sp. Inoculation with *P. striatia* or *B. polymyxa* and *Rhizobium* sp. also led to 39 to 103% greater nitrogenase activity (ARA) at 45 and 90 DAP. Inoculation of chickpea with either of the suspected PGPR strains alone resulted in 49 to 51% greater nodule dry weights versus uninoculated plants at 45 DAP. Such inoculation also resulted in 367 to 433% increases in nitrogenase activity (ARA) at 45 and 90 DAP. The authors attributed the promotions found to increased solubilization of P or the production of growth promoting substances by the test strains used.

Li and Alexander (1988) isolated antibiotic-producing *Bacillus* and *Pseudomonas*

sp. strains. In greenhouse experiments using field soil, inoculation of alfalfa (*Medicago sativa* L., cv. Oneida VR) with either the *Bacillus* sp. or *Pseudomonas* sp. strain and an antibiotic-resistant *Rhizobium meliloti* strain resulted in 48 to 71% greater root nodule numbers versus use of the *R. meliloti* strain alone. Soybean (cv. Evans) inoculated with the *Bacillus* sp. strain and an antibiotic-resistant *B. japonicum* strain had 55 to 57% greater nodule numbers than soybean inoculated with only the *B. japonicum* strain. The authors concluded that the establishment of *R. meliloti* and *B. japonicum* had been restricted by competition from other indigenous rhizobacteria. The antibiotic- producing PGPR strains eliminated these antagonists from the rhizosphere allowing the resistant *R. meliloti* and *B. japonicum* strains to become better established and form more nodules.

In similar work, Knight and Langston-Unkefer (1988) inoculated alfalfa plants with a toxin-releasing *Pseudomonas syringae* pv. *tabaci* strain and *R. meliloti* in laboratory experiments using sterilized sand as the growth medium. At 30 DAP, plants that received *P. syringae* pv. *tabaci* had 122% greater root nodule numbers, 123% greater nodule weights and 48% greater nitrogenase activity compared to plants inoculated with only *R. meliloti*. At 45 DAP, root nodule numbers were 141% greater and nitrogenase activity was 194% greater for plants that received *P. syringae* pv. *tabaci*. Knight and Langston-Unkefer (1988) estimated that plants inoculated with the suspected PGPR were provided with five times the total potential nitrogenase activity of controls. The authors proposed that the toxin released by the *P. syringae* pv. *tabaci* strain impaired glutamine synthetase-catalyzed ammonia assimilation in nodules. This resulted in altered glutamate and glutamine pools in plant nodules and roots which influenced N_2 fixation, N

assimilation and nodule formation.

Derylo and Skorupska (1993) found that clover seedlings (*Trifolium pratense* L. cv. Hruszowska) inoculated with *Pseudomonas* sp. strain 267 and one of three *R. leguminosarum* bv. *trifolii* strains and grown under gnotobiotic conditions for five weeks displayed 49% greater nodule fresh weights and 66 to 185% greater nitrogenase activity (ARA) compared to plants inoculated with the *R. leguminosarum* bv. *trifolii* strains alone. *Pseudomonas* sp. strain 267 did not promote the growth of non-nodulated clover plants provided with nutrient solution. Derylo and Skorupska (1993) were also able to show that *Pseudomonas* sp. strain 267 excreted the water soluble vitamins thiamin and panthotenate and that addition of these vitamins to nodulated clover plants produced nodulation and nitrogenase activity responses similar to results seen with the suspected PGPR.

Chanway et al. (1989) screened seven *P. putida* and *P. fluorescens* strains for their ability to promote the nodulation and nitrogen fixation of lentil and field pea in a series of sterile and non-sterile laboratory and field studies. None of the strains used demonstrated any PGPR abilities with field pea (cv. Trapper). In sterile laboratory experiments using the Leonard jar system (Vincent 1970), lentil (cv. Eston) inoculated with *P. putida* strain G2-8 and *Rhizobium leguminosarum* bv. *viceae* strain 175P1 demonstrated 15% greater root nodule numbers compared to lentil inoculated with the *R. leguminosarum* bv. *viceae* strain alone. No increases in nitrogenase activity (ARA) were found. Lentil (cv. Laird) displayed no responses to G2-8 inoculation. In tests using sand columns, use of strain G2-8 resulted in 54% greater nitrogenase acitivity for the cultivar Eston lentil and 15%

greater nitrogenase activity for the cultivar Laird lentil, compared to either lentil cultivar inoculated with the *R. leguminosarum* bv. *viceae* strain alone. Root nodule numbers were unaffected. The cultivar Eston, inoculated with *P. putida* strains G2-8 or G11-32 and grown in non-sterile potting soil displayed no increases in nitrogenase activity or root nodule numbers . The cultivar Laird grown in the same manner demonstrated a depression in nitrogenase activity at 14 DAP but were found to match control plant levels at 21 DAP. In field experiments, inoculation of the cultivar Eston with strain G11-32 led to 46% greater root nodule numbers and 228% greater nitrogenase activity compared to inoculation with the *R. leguminosarum* bv. *viceae* strain alone. Inoculation of the cultivar Eston with strain G2-8 led to 42% greater root nodule numbers while inoculation with *P. fluorescens* strain G12-22 produced 130% greater nitrogenase activity. The authors felt that since growth promotions were seen under both sterile and non-sterile conditions, the suppression of pathogens was not the cause of these promotions. Further, it was noted that different PGPR modes of action may have been at work for each of the lentil cultivars used.

Turner and Backman (1991) conducted a series of multi-environment studies involving *Bacillus subtilis* strain A-13 applied to peanut. In one instance during a series of replicated field studies conducted from 1983 to 1985, use of the strain resulted in a nodulation rating of 4.3 compared to 3.5 for peanut not inoculated with *B. subtilis* strain A-13. The authors suggested that growth promotions may have been due to *B. subtilis* strain A-13 increasing peanut root growth thereby creating more potential nodulation sites or improving plant nutrition.

Srinivasan et al. (1996) isolated several *Bacillus* sp. strains from common bean rhizospheres and screened the strains for their ability to produce IAA. Common bean (cv. Contender), grown under gnotobiotic conditions using the Leonard jar system, were co-inoculated with each of the test strains and *Rhizobium etli* strain TAL 182. At 24 DAP, use of the test strains resulted in 37 to 87% greater root nodule numbers and 33 to 83% greater nodule fresh weights than that found for common bean inoculated with the *R. etli* strain alone. Nitrogenase activity (ARA) was also found to be 76 to 115% greater due to use of the *Bacillus* sp. strains. In vitro tests of IAA production capabilities of the test strains did not correlate closely with the growth promotions observed.

Using sterile growth pouches, Srinivasan et al. (1997) tested the ability of *B. megaterium* strain S49 to enhance the nodulation of common bean by *R. etli* strain TAL 182. At 14 DAP, inoculation with strain S49 had resulted in 38% more root nodules compared to inoculation with the *R. etli* strain alone. Nodulation also occurred more quickly. Common bean inoculated with strains S49 and TAL 182 showed peak nodulation at 11 DAP versus 13 DAP for common bean inoculated with only TAL 182. It was also found that promotions in root nodule numbers and nodule fresh weights only occurred in the presence of live *B. megaterium* strain S49 cells. In a split root experiment it was found that inoculation of common bean with S49 and TAL 182 on the primary side of the split root reduced the percent suppression of nodulation on the secondary side of the split root. Based on their evidence, the authors suggested that their test strain was either promoting the development of root hairs and so increasing possible sites for nodulation or else partially overcoming plant host-mediated autoregulation of

nodulation.

Table 2.2 summarizes some of the PGPR-mediated promotions of the (Brady)Rhizobia-legume symbiosis discussed in this review. It is important to note that responses varied greatly across plant species. This occurred even when species had been tested with the same PGPR. PGPR-mediated responses ranged from being non-existent to being substantial in size.

Table 2.2: Summary of PGPR-mediated (Brady)Rhizobia-legume symbiosis responses (+/- % change) discussed in the literature review.

Crop	PGPR	Nodule Number (%)	Nodule Weight (%)	Nitrogenase Activity (%)	Reference
Soybean	*A. brasilense*	+9956	+32 to +667	-	Singh and Subba Rao (1979)
Peanut	*A. lipoferum*	+47	+31 to +70	-	Raverkar and Konde (1988)
Garden Pea	*A. brasilense*	+19	-	None	Sarig et al. (1986)
Vetch	*A. brasilense*	None	-	+113	Sarig et al. (1986)
Sulla Clover	*A. brasilense*	None	-	+74	Sarig et al. (1986)
Soybean	*A. vinelandii*	+14 to +495	-	None	Burns et al. (1981)
Cowpea	*A. vinelandii*	+40 to +49	-	None	Burns et al. (1981)
Sweet Clover	*A. vinelandii*	+123 to +138	-	None	Burns et al. (1981)
White Clover	*Azospirillum* sp.	+25 to +100	-	None	Plazinski and Rolfe (1985a)
Bur Clover	*A. brasilense*	+30	-	+392 to +1000	Yahalom et al. (1987)
Siratro	*A. brasilense*	-	-	+25 to +80	Yahalom et al. (1987)
Common Bean	*P. putida*	+15 to +300	-	-	Grimes and Mount (1984)
Soybean	*Pseudomonas* sp.	+42 to +164	+34 to +371	-	Polonenko et al. (1987)
Chickpea	*P. striatia* *B. polymyxa*	+41 to +50	+49 to +51	+39 to +433	Alagawadi and Gaur (1988)
Alfalfa	*Bacillus* sp. *Pseudomonas* sp.	+48 to +71	-	-	Li and Alexander (1988)
Soybean	*Bacillus* sp. *Pseudomonas* sp.	+55 to +57	-	-	Li and Alexander (1988)
Alfalfa	*P. syringae*	+122 to +141	+123	+48 to +194	Knight and Langston-Unkefer (1988)
Clover	*Pseudomonas* sp	-	+49	+66 to + 185	Derylo and Skorupska (1993)
Field Pea	*Pseudomonas* sp.	None	None	None	Chanway et al. (1989)
Lentil	*P. putida*	None to +46	-	None to +228	Chanway et al. (1989)
Common Bean	*Bacillus* sp.	+37 to +87	+33 to +83	+76 to +115	Srinivasan et al. (1996)

2.3 Promotion of Seed Yield, the Growth of Plant Parts, and Nutrient Content

When examining the work done on PGPR, it becomes obvious that promotions in growth and yield have been the focal point of attention. Historically, as shown by Kloepper et al. (1989) in examining a review by Brown (1974), pioneering work in the Soviet Union focussed on yield increases. Kloepper et al. (1989) noted that after 1974, the focus of bacterial inoculant work shifted towards the biological control of diseases. But Glick (1995) has stated that over the previous 10 to 15 years, along with an increased level of understanding of PGPR mechanisms, has come renewed interest in PGPR. This has come, in no small part, to the fact that many PGPR may have the potential to become marketable products (eg: Okon 1985; Okon and Hadar 1987; Turner and Backman 1991; Mahafee and Backman 1993). It is reasonable to suggest that potential users of these products would look at yield increases of marketable plant parts as measures of how useful these products are. It is important to note the observations of Kloepper et al. (1989) that while bacterial inoculants could increase crop yield, inconsistent performance remains a major hurdle in the development of commercial formulations. They attributed this problem to the complexity of the system which determines yield because this system functions as a set of multiple interactions between the introduced bacteria, crop plants, the soil microflora and several environmental variables. As in the previous section, literature examined will be organized along genera lines starting with *Azospirillum* and moving to *Pseudomonas* and *Bacillus*.

Singh and Subba Rao (1979), in work described previously, demonstrated 66% greater shoot dry weights for soybean inoculated with *A. brasilense* strain Madhu and

86% greater shoot dry weights for soybean inoculated with *A. brasilense* strain Sp. 7 compared to uninoculated control plants. The authors noted that increases in shoot dry weight persisted as soil N content was increased when *A. brasilense* strain Madhu was used.

In greenhouse experiments described previously, Sarig et al. (1986) found that vetch plants inoculated with a mixture of *A. brasilense* strains had 23% greater shoot dry weights, 15% greater root dry weights and 14% greater shoot N concentrations than uninoculated control plants at 12 weeks after planting. Six weeks after planting, garden pea did not exhibit any increases in plant mass or N concentrations attributable to inoculation with the *A. brasilense* mixture. In field experiments described previously, Sarig et al. (1986) found that chickpea plants inoculated with the *A. brasilense* strain mixture had 17% greater seed yields compared to uninoculated control plants. Garden pea inoculated with the strain mixture demonstrated 9% greater seed yields and a 29% increase in the number of pods plant^{-1}. The inoculation of vetch with the *A. brasilense* mixture led to 23% greater shoot DM and 17 % greater shoot N content.

Raverkar and Konde (1988), in a field experiment described previously, found that whole plant N concentrations were 29% greater in Robut 33-1 peanut inoculated with *A. lipoferum* strain ICM 1001 compared to uninoculated plants at 60 DAP. At harvest, Robut 33-1 peanut inoculated with the *A. lipoferum* strain demonstrated 24% greater pod yields, 21% greater whole plant DM and 68% greater shoot N contents compared to uninoculated plants. As well, JL 24 peanut inoculated with the *A. lipoferum* strain alone demonstrated 35% greater pod yields, 21% greater whole plant DM and 86%

greater shoot N contents compared to uninoculated plants.

Grimes and Mount (1984), in work described previously, found only one instance, over two years of experimentation, where the use of the antibiotic-producing *Pseudomonas putida* strain M-17 increased shoot fresh weights of common bean in the field. The authors characterized this increase as an isolated occurrence.

Polonenko et al. (1987), in greenhouse experiments described previously, demonstrated that promotions of soybean shoot and root dry weights in a soil-less planting mix were dependent on the combination of *P. fluorescens* or *P. putida* and *B. japonicum* strains used. With *B. japonicum* strain USDA 110, use of the *P. putida* strain RC1 resulted in 38% greater shoot dry weights compared to plants inoculated with *B. japonicum* strain USDA 110 alone. With *B. japonicum* strain USDA 118, use of the *P. putida* strains RC2 and RC12 and the *P. flourescens* strains RC1 and RC7 resulted in 19 to 34% greater shoot dry weight compared to plants inoculated with *B. japonicum* strain USDA 118 alone. As well, the use of the *P. putida* strains RC2 and RC4 and the *P. flourescens* strains RC1, RC6 and RC14 with *B. japonicum* strain 118 led to 52 to 79% greater root dry weight. Test strains which caused promotions in shoot and root dry weights rarely caused promotions in root nodule number or nodule dry weight.

Alagawadi and Gaur (1988), in work described previously, found that inoculation of chickpea with a *B. polymyxa* strain alone resulted in 41% greater whole plant DM at 90 DAP and 21% greater grain yields compared to uninoculated control plants. Further, grain N content was 20% greater and grain P content was 22% greater for chickpea inoculated with the *B. polymyxa* strain. Inoculation of chickpea with a *P. striata* strain

alone resulted in 29% greater whole plant DM compared to uninoculated plants at 90 DAP. As well, grain N content was 16% greater and grain P content was 22% greater for chickpea inoculated with the *P. striata* strain. While co-inoculation of chickpea with the test strains and *Rhizobium* sp. strain F75 resulted in no whole plant DM or grain yield increases, grain P content was 10 to 20% greater compared to plants inoculated with only the *Rhizobium* sp. alone. As well, inoculation with the *B. polymyxa* strain and *Rhizobium* sp. strain F75 led to 7% greater grain N content than found for chickpea inoculated with the *Rhizobium* sp. alone.

Li and Alexander (1988), in work described previously, found that inoculation of soybean with their antibiotic-producing *Bacillus* sp. strain and an antibiotic-resistant *B. japonicum* strain resulted in 86% greater pod numbers plant^{-1}, 16% greater fresh seed weights, 13% greater pod dry weights and 42% greater whole plant dry weights compared to soybean inoculated with *B. japonicum* alone. These promotions were found in only one of a series of similar studies conducted by the authors. Promoitons of nodulation did not always correlate with promotions in seed yields or dry weight accumulations.

Knight and Langston-Unkefer (1988), in work described previously, found that alfalfa seedlings inoculated with a toxin-releasing *Pseudomonas syringae* pv. *tabaci* strain and *R. meliloti* had whole plant dry weights that were 95% greater than plants inoculated with *R. meliloti* alone. At 45 DAP, foliar fresh weights were 83% greater while root and nodule fresh weights were 224% greater for alfalfa inoculated with both bacterial strains versus alfalfa inoculated with *R. meliloti* alone. As well, whole plant N contents were 108% greater for plants inoculated with *R. meliloti* and the *P. syringae* pv.

tabaci strain.

Levesque et al. (1993) found that inoculation of alfalfa cv. Saranac with *Pseudomonas fluorescens* strain 61-9A and *Rhizobium meliloti* strain S14 led to 38% greater shoot DM compared to alfalfa inoculated with only the *R. meliloti* strain. As well, inoculation of alfalfa with *Pseudomonas putida* strain G2-8 and *Rhizobium meliloti* strain S14 led to 32% greater shoot DM. Plants were grown in a greenhouse using an autoclaved soil:sand:vermiculite planting medium. No grain yield increases were seen at the end of the growing season and growth promotions did not occur in experiments where the planting medium was not autoclaved.

Chanway et al. (1988) inoculated compartmentalized flats of soil with treatments including a *B. polymyxa* strain with or without a *R. leguminosarum* bv. *trifolii* strain. White clover plants grown in soil inoculated with the *B. polymyxa* stain and *R. leguminosarum* bv. *trifolii* had greater whole plant dry weights than plants grown in soil inoculated with only *R. leguminosarum* bv. *trifolii*. Increasing the level of homology in genetics among clover plants and between clover plants and the *B. polymyxa* strain used, increased the size of whole plant dry weight promotions. Partial homology resulted in a 23% increase in plant dry weights while substantial homology resulted in a 43% increase in plant dry weights. This work was cited as evidence that PGPR enhancements of plant growth could depend on the genetics of the host plant and the PGPR being used.

Chanway et al. (1989), in work described previously, found that inoculation of lentil (cv. Laird) with *P. putida* strain G2-8 and *R. leguminosarum* bv. *viceae* strain 175P1 resulted in 27% greater shoot dry weights and 93% greater root dry weights at 35

DAP than lentil inoculated with the *R. leguminosarum* bv. *viceae* strain alone. Plants had been grown in a growth chamber under non-sterile conditions. Lentil (cv. Eston) demonstrated no growth promotions using the same experimental procedure. In a series of sterile experiments involving Leonard jars, the cultivar Eston inoculated with *P. putida* strains G2-8 or G11-32 and *R. leguminosarum* bv. *viceae* and the cultivar Laird inoculated with strain G2-8 and *R. leguminosarum* bv. *viceae* did not demonstrate any promotions in shoot or root dry weights compared to lentil inoculated with the *R. leguminosarum* bv. *viceae* alone. In a series of sterile experiments using N-free sand columns, inoculation of the cultivar Eston with *P. putida* strain G2-8 and *R. leguminosarum* bv. *viceae* produced 46% greater shoot dry weights and 51% greater root dry weights compared to lentil inoculated with *R.. leguminosarum* bv. *viceae* alone. Inoculation of same cultivar with *P. putida* strain G11-32 resulted in 36% greater root dry weights. As well, inoculation of the cultivar Laird with *P. putida* strain G2-8 resulted in 22% greater shoot dry weights than lentil inoculated with *R. leguminosarum* bv. *viceae* alone. Chanway et al. (1989) noted that lentil cultivar was an important factor in determining the responses to the PGPR strains used.

Turner and Backman (1991), in work described previously, demonstrated an 11.7 % increase in seed yield in 1983 and a 17% increase in seed yield in 1985 due to the inoculation of peanut with *Bacillus subtilis* strain A-13 in five replicated field studies conducted between 1983 and 1985. In 24 unreplicated field trials, yield increases due to strain A-13 ranged from -3.5 to 37% with an average 7.6% yield increase across all sites. In these trials, peanut planted earliest into fields where legumes had been grown within

the last two years seemed to display the greatest yield responses to *B. subtilis* strain A-13. In the 1985 replicated field study, the upper leaves, lower leaves and stems of plants inoculated with strain A-13 had 15%, 11% and 12% greater N contents, respectively than the upper and lower leaves and stems of uninoculated plants.

In a rhizotron study involving peanut grown with or without the application of moisture stress, Turner and Backman (1991) found that pod numbers plant^{-1} were increased 6% through use of *B. subtilis* strain A-13 regardless of the irrigation regime. Under moisture stressed conditions, strain A-13 also increased the number of pegs formed by 100% compared to uninoculated control plants.

Liu and Sinclair (1990) found that soybean grown from seeds coated with either *Bacillus megaterium* strain ATCC-55000 or M2144 had greater whole plant dry weight than controls in controlled-environment studies. Liu and Sinclair (1990) concluded that the mechanism involved in growth promotion was not the same as that which enabled strain ATCC-55000 to antagonize *Rhizoctonia solani* because strain M2144 was an antagonist-deficient mutant.

In greenhouse experiments using sterilized field soil, Azcon (1993) inoculated sulla clover (*Hedysarum coronarium* L.) with *Rhizobium* sp. plus one of three different components of a *Pseudomonas* sp. culture (washed cells, cell-free filtrates or complete bacterial culture) or a mixture of phytohormones in the presence or absence of the VAM inoculum of *Glomus mosseae*. Azcon (1993) found that application of any component of the *Pseudomonas* sp. culture to mycorrhizal plants at one DAP resulted in 124 to 154% greater whole plant DM compared to control plants at ten weeks after planting. Non-

mycorrhizal plants demonstrated no growth promotions. As well, the application of any component of the *Pseudomonas* sp. culture to mycorrhizal plants at one DAP resulted in up to 26% greater shoot N contents and 31 to 59% greater shoot P contents compared to control plant shoots harvested at ten weeks after planting. In non-mycorrhizal plants, inoculation with the *Psuedomonas* sp. whole bacterial culture at one DAP resulted in 111% greater shoot N contents at 10 weeks after planting. Shoot K contents was 7 to 33% greater in mycorrhizal plants that were inoculated with any component of the *Pseudomonas* sp. culture. Inoculation with the cell-free filtrate resulted in 17% greater shoot Ca contents and 69% greater shoot Mg contents. In non-mycorrhizal plants, inoculation with the cell-free filtrate led to 96% greater shoot K contents while inoculation with whole bacterial culture resulted in 97% greater shoot K contents. Inoculation with washed cells led to 74% greater shoot Ca contents and inoculation with whole bacterial culture resulted in 54% greater shoot Ca contents. Azcon (1993) concluded that bacterial culture fraction used played a significant role in determining the effectiveness of PGPR-VAM-*Rhizobium* sp. associations.

Derylo and Skorupska (1993), in work described previously, demonstrated that inoculation of while clover plants with *Pseudomonas* sp. strain 267 and one of four *Rhizobium leguminosarum* bv. *trifolii* strains resulted in 25 to 58% increases in whole plant fresh weights compare to plants inoculated with the *R. leguminosarum* bv. *trifolii* strains alone. The authors did not observe similar growth promotions when clover plants were inoculated with strain 267 alone.

Table 2.3 summarizes the PGPR-mediated seed yield and plant part growth

responses discussed in this review. It is important to note that responses varied greatly across plant species. This occurred even when species had been tested with the same PGPR. Both positive and negative PGPR-mediated responses were evident.

Table 2.3: Summary of PGPR-mediated seed yield and plant part growth responses (+/- % change) discussed in the literature review.

Crop	PGPR	Whole Plant (%)	Stems, Leaves and Pods (%)	Root (%)	Seed (%)	Reference
Soybean	*A. brasilense*	-	+66 to +86	-	-	Singh and Subba Rao (1979)
Vetch	*A. brasilense*	-	+23	+15	-	Sarig et al. (1986)
Garden Pea	*A. brasilense*	-	+29	-	None to +9	Sarig et al. (1986)
Chickpea	*A. brasilense*	-	-	-	+17	Sarig et al. (1986)
Peanut	*A. lipoferum*	+21	+24 to +35	-	-	Raverkar and Konde (1988)
Soybean	*P. putida* *P. flourescens*	-	+19 to +38	+52 to +79	-	Polonenko et al. (1987)
Chickpea	*B. polymyxa*	None to +41	-	-	None to +21	Alagawadi and Gaur (1988)
Chickpea	*P. striata*	None to +29	-	-	None	Alagawadi and Gaur (1988)
Soybean	*Bacillus* sp.	+42	+13	-	+16	Li and Alexander (1988)
Alfalfa	*P. syringae*	+95	+83	+224	-	Knight and Langston-Unkefer (1988)
Alfalfa	*P. flourescens*	-	+38	-	None	Levesque et al. (1993)
Alfalfa	*P. putida*	-	+32	-	None	Levesque et al. (1993)
White Clover	*B. polymyxa*	+23 to +43	-	-	-	Chanway et al. (1988)
Field Pea	*P. putida*	-	None	None	-	Chanway et al (1989)
Lentil	*P. putida*	-	None to +46	None to +93	-	Chanway et al (1989)
Peanut	*B. subtilis*	-	-	-	-3.5 to +37	Turner and Backman (1991)
Sulla Clover	*Pseudomonas* sp.	+124 to +154	-	-	-	Azcon (1993)
White Clover	*Pseudomonas* sp.	+25 to +58	-	-	-	Derylo and Skorupska (1993)

2.4 Current Research Status of *Bacillus cereus* strain UW85

Bacillus cereus strain UW85 (here on referred to as UW85) was isolated from a group of bacterial strains found on alfalfa roots (Handelsman et al. 1988). UW85 was part of a laboratory screening trial where isolates were being assessed for their ability to protect alfalfa seedlings from damping-off caused by *Phytophthora megasperma* f.sp. *medicaginis*. In other screening trials, UW85 was found to protect alfalfa from damping-off caused by *Pythium* sp. and *Aphanomyces* sp. and also protected soybean from damping-off caused by *Phytophthora* sp. In field experiments, alfalfa and soybean seeds were coated with a UW85 spore paste and planted into a poorly drained field during periods of cool soil temperatures. UW85 increased the seedling emergence of both alfalfa and soybean by 50%.

Handelsman et al. (1990) found that filtrates of sporulating cultures of UW85 provided as much disease protection to plants as whole cultures. No plant protection ability was found when plants were inoculated with filtrates of vegetative cultures or cultures containing endospores. The authors speculated that the biologically active material produced by UW85 was released into culture during the release of spores and that this material was a unique product of UW85 sporulation. Handelsman et al. (1990) were able to separate these cultures into two fractions which actively protected plants at different pH levels. A non-protecting mutant of UW85 was also isolated. Field testing of UW85 as a spore paste applied to alfalfa seeds resulted in a 67% increase in seedling emergence compared to uninoculated control seeds.

Handelsman et al. (1991) demonstrated that sporangia of *Phytophthora parastica*

pv. *nicotianae* were not produced on infected tobacco seedlings which had been simultaneously inoculated with pathogen zoospores and a sporulated culture of UW85. Phipps (1992) demonstrated that sporulated cultures of UW85 were able to protect peanut from Sclerotinia blight caused by *Sclerotinia minor*. Smith et al. (1993) found that fully sporulated cultures of UW85 were effective in the suppression of cucumber fruit rot, commonly called cottony leak, caused by *Pythium aphanidermatum*. Silo-Suh et al. (1994) were able to purify two antibiotics produced by UW85 which reversibly inhibited the growth of *P. medicaginis*. One was designated zwittermicin A and was found to an aminopolyol of 396 Da. The second was designated antibiotic B and was found to be an aminoglycoside containing a disaccharide. As well, a mutant strain of UW85 which did not possess disease suppressive abilities was isolated.

Gilbert et al. (1993) examined the effects on rhizosphere population dynamics of introducing UW85 into bacterial communities on the roots of soybean both in growth chamber and field studies. They were able to show that the bacterial composition of soybean rhizosphere soil was different from non-rhizosphere soil in that species that were resistant to a wider range of anti-microbial compounds were favoured in rhizosphere soil. However, the effects of such introductions were variable and growth chamber studies did not reflect what was found in the field.

The ability of UW85 to enhance the nodulation of soybean in the field and in a growth chamber was first explored by Halverson and Handelsman (1991). In the growth chamber, soybean (cv. AP200) were coated with either a paste of UW85 endospores alone, a paste of endospores derived from the non-biologically active mutant, a paste of

either group of endospores mixed 1:1 with methylcellulose or methylcellulose alone. Seeds were planted in glass tubes filled with autoclaved soil:vermiculite mixture and inoculated with *B. japonicum* strain 110 at planting. Three different soils (Joy silt-loam, acid-washed sand and Plainfield sand) were used and the plants were allowed to grow for 28 days. Halverson and Handelsman (1991) found that UW85 seed inoculation increased the number of root nodules per plant by 34 to 61% compared to untreated controls. In the Joy silt-loam, nodule mass was increased by 33% through UW85 inoculation. In the Plainfield sand, nodule mass increases due to UW85 inoculation ranged from 13 to 70% although these increases were not significant at P=0.05. Plants treated with the spore paste produced from the non-biologically active mutant strain displayed no nodulation enhancements. Nitrogenase activity (ARA), was 25 to 73% greater for UW85 treated soybean as compared to untreated controls.

Three field experiments were conducted over three years using seeds coated with UW85 spore paste (Halverson and Handelsman 1991). In 1986, UW85-treated soybean had greater mean nodule root number per plant (19.5) than plants treated with methylcellulose alone (14.3). In 1987 and 1989, nodulation of UW85-treated plants was 87 to 134% greater than in untreated plants at 28 DAP. By 35 DAP, UW85-treated plants had 31 to 39% more root nodules than untreated plants. It was noted that UW85 increased the nodulation of both primary and lateral roots compared to controls. By 49 DAP, differences among treatments for root nodule numbers no longer existed in all three field experiments. Halverson (1991), in reference to the field experiments, noted that seedling emergence was increased 33 to 40% by UW85 in 1987. Seed yields in 1987

were 38 to 50% greater for UW85-treated soybean at P=0.10 while no differences among treatments were found in the 1989 experiment. In 1989, seedling fresh weight was greater for UW85-treated plants compared to controls on the first, second and fifth DAP. Radicle growth on the fifth and seventh DAP was also found to be greater for UW85-treated plants. In 1987 increases in root length for UW85-treated plants were found at 35 and 49 DAP. As well, increases of 22 to 34% in shoot height for UW85-treated plants versus control plants were found. Halverson (1991) noted that, in all the field experiments done, disease incidence was very low regardless of treatment.

In a series of field experiments by Osburn et al. (1995), UW85 in several spore-based formulations was evaluated for its effect on yield when applied to three soybean cultivars at two Wisconsin field sites (Racine and Whitewater) over a five year period beginning in 1989. *Phytophthora sojaie* was naturally present in the fields and low to severe potential for damping-off and root rot existed every year. One of the soybean cultivars used had genetic resistance to infection by *P. sojae* while another was considered tolerant and the third was considered susceptible to the disease.

At the Racine site, use of a in-furrow clay granule formulations resulted in 139%, 15% and 14% greater seed yields for the susceptible cultivar compared to controls in 1990, 1991 and 1992, respectively (P=0.10). A seed-applied peat powder formulation increased yield 18% in 1989 while a seed-applied liquid formulation resulted in 33% greater yield in 1993. An in-furrow clay granule formulation of UW85 produced 31 and 10% greater yields with the tolerant cultivar in 1990 and 1992, respectively and 47 and 31% greater yields for the resistant cultivar in 1990 and 1991, respectively.

Despite claims of significant effects at P=0.10 by Osburn et al. (1995), a re-examination of the data at P=0.05 using LSD values provided by the author of this thesis revealed that only one seed yield promotion above the level of the untreated control was found. In 1990, an in-furrow clay granule formulation of UW85 used with the susceptible cultivar resulted in 139% greater yield.

At their Whitewater site, Osburn et al. (1995) found a 14% increase in seed yield for the susceptible cultivar when UW85 was used as a seed-applied clay powder formulation in 1990 (P=0.10). Yield of the tolerant cultivar was 16% greater in 1991 and 1992 through use of an in-furrow clay granule formulation. For the resistant cultivar, yields were 15%, 19% and 12% greater in 1990, 1991 and 1992, respectively due to the use of a seed-applied clay powder formulation. As well, in-furrow applied clay granule formulations resulted in 10 to 23% greater yields with the resistant cultivar in 1992. Also in 1992 with the resistant cultivar, an in-furrow applied liquid formulation led to 16% greater yield while a seed-applied wettable powder formulation resulted 17% greater yield.

Again, a re-examination of the data at P=0.05 using LSD values for yield provided by the author of this thesis revealed that, in 1990, a seed-applied clay powder formulation of UW85 increased the seed yield of the susceptible cultivar by 14% and also increased the yield of the resistant cultivar by 15%. In 1991, 19% greater yield was found when the resistant cultivar received a seed-applied clay powder formulation of UW85. In 1992, the yield of the tolerant cultivar was 16% greater when an in-furrow applied clay granule formulation of UW85 was used. Also in 1992, yields of the resistant

cultivar were 10 to 23% greater due to the use of in-furrow applied clay granule formulations. As well, resistant cultivar yield in 1993 was 17% greater when a liquid formulation of UW85 was used and 16% greater when a seed-applied wettable powder formulation was used.

Osburn et al (1995) also recorded stand counts at Racine and Whitewater in 1990 at 14 and 28 DAP. At Racine, the application of an in-furrow applied clay granule formulation of UW85 resulted in 82% greater emergence for the susceptible cultivar at 28 DAP (P=0.05). At Whitewater, most of the UW85 formulations used led to emergence increases for all three soybean cultivars that ranged from 27 to 108% at 14 DAP and from 22 to 96% at 28 DAP (P=0.05). The authors concluded that emergence promotions due to UW85 seen at the Racine site were due to control of Phytophthora damping-off disease. Because many UW85-mediated seed yield and plant stand density promotions seen at the Whitewater site occurred with the resistant cultivar, Osburn et al. (1995) attributed these effects to factors other than disease control.

In this review of the literature pertaining to PGPR effects with legumes, three important categories of plant growth promotion have been examined. The first of these was the ability of PGPR to enhance seedling emergence an vigour. The second category was the ability of PGPR to promote the (Brady)Rhizobia-legume symbiosis. The third category was the ability of PGPR to promote seed yield, the growth of plant parts and nutrient content. In all three categories, experimental evidence was found to show that when specific PGPR were used with specific plant species or even specific cultivars, plant growth promotions did occur. Evidence for the PGPR phenomenon was found in

laboratory, growth chamber, greenhouse and field experiments. Research investigating the possibility of the *Bacillus cereus* strain UW85 being a PGPR was also examined. Evidence of the strain's ability to act as a bio-control for certain plant diseases was found in both laboratory and field experiments. Putative evidence, from both laboratory and field experiments, of UW85's ability to promote various parameters of soybean growth, yield and the *Bradyrhizobium*-legume symbiosis was found. However, this work addressed only one species in situations where it was often unclear how large a role disease control played in the enhancements and promotions observed.

3.0 Materials and Methods

3.1 Controlled-Environment Studies

Experiments were conducted to monitor the effects that treating soybean seed (*Glycine max* L. Merr. cv. Maple Ridge), common bean seed (*Phaseolus vulgaris* L. cv. OAC Rico) and field pea seed (*Pisum sativum* L. cv. Express) with a spore paste derived from *Bacillus cereus* strain UW85 (here on referred to as UW85) would have on plant growth and N_2 fixation. Four experiments were conducted under controlled- environment conditions, one each with soybean and common bean and two with field pea at different temperature regimes. In each experiment, treatments included seed treated with or without UW85 and one of two rhizobia concentrations plus appropriate controls.

3.1.1 *Seed Coating and Inoculant Preparation*

A spore paste of *B. cereus* strain UW85 was produced following the methodology outlined by Halverson and Handelsman (1991). Strain UW85 was a gift of J. Handelsman, University of Wisconsin, Madison. Trypticase soy agar (TSA, BBL Cockeysvile, MD) plates were inoculated with 1.0 ml of a mid-log phase culture of *B. cereus* UW85 grown in half-strength Trypticase soy broth (TSB, BBL Cockeysvile, MD). Plates were incubated for 96 hours at 28°C. A lawn of spores grew across the agar surface over the incubation which was then scraped off the agar surface using a sterile razor blade to form the spore paste.

All seed in each experiment was treated with a 0.26% sodium hypochlorite solution for 5 minutes, rinsed with distilled water and then spread out in a single layer in

open sterile petri dishes and allowed to dry. Seeds to be coated were placed into sterile plastic pouches (Fisher Scientific, Winnipeg, MB) along with the spore paste and shaken vigorously until all seeds appeared evenly coated with paste (25 seeds per pouch). Seeds were then removed from the pouches and placed in a single layer in open sterile petri dishes where they were allowed to dry. Fifty seeds were prepared in this fashion. All operations were carried out in a laminar flow hood.

After the coated seeds had dried, five were randomly selected from the prepared population and each was individually placed in a 50 ml Erlenmeyer flask containing 25 ml of sterile distilled water. The flasks were then sonicated for one minute each and one ml from the resulting suspensions was serially diluted in sterile distilled water. The dilutions were plated on half-strength Trypticase soy agar and incubated at 28°C for 24 hours. The resulting colony forming units were counted. For soybean, the UW85 spore paste seed treatment resulted in 1.4×10^7 to 2.1×10^7 CFU seed^{-1} while for common bean, this treatment resulted in 1.8×10^6 to 9.6×10^6 CFU seed^{-1}. In the case of field pea, the UW85 spore paste treatment resulted in 6.7×10^6 to 1.9×10^7 CFU seed^{-1}.

In all experiments, as well as seed being either with or without the UW85 coating (+B or -B), seed could also either be inoculated with a high (HR) or a low (LR) concentration of the appropriate rhizobia at the time of planting. The high concentration inoculum consisted of bacteria numbering in the 10^6 range of CFU while the low concentration inoculum consisted of bacteria numbering in the 10^1 range of CFU. For the experiment involving common bean, the high and low concentrations were 10^7 and 10^2 CFU, respectively. The methods used to prepare these inoculants were as follows. All

cultures were grown in full strength yeast mannitol broth (YMB) for 120 hours at 28°C. Pure cultures were then serially diluted twice in a buffered solution (1.0g peptone, 0.34g KH_2PO_4 and 1.21 g K_2HPO_4 in 1 L of distilled water). One sample of 0.5 ml from each to the 10^{-2} dilutions was enumerated using a Petroff-Hauser hemocytometer. To facilitate counting, 0.5 ml of 10% formalin solution was added to each of the dilution samples to impede locomotion by the bacteria. Several comparisons of hemocytometer and serial dilution plating were conducted previous to the experiments and had shown that both methods repeatedly gave concentrations within the same log unit. When experiments were done, only the hemocytometer was used to determine bacteria concentrations. In the case of soybean, the pure culture of *Bradyrhizobium japonicum* strain USDA 138 used was found to be at a concentration of 3.8×10^9 cells ml^{-1}. For common bean, the pure culture of *Rhizobium etli* strain TAL 182 used was found to be at a concentration of 3.4×10^9 cells ml^{-1} while for field pea the *Rhizobium leguminosarum* bv. *viceae* strain 128A1 used was found to be at a concentration of 4.2×10^9 cells ml^{-1}. All strains used were Hup^-. Once the cell concentrations of the pure cultures were known, the dilutions containing the needed concentration of cells were bulked and loaded into sterile syringes equipped with sterile needles. All of these operations, except for the actual hemocytometer counts, were conducted under a laminar flow hood.

The syringes containing the rhizobia were then immediately transported to a growth cabinet housing an experiment and were applied to the planted but still uncovered seeds at a rate of 1 ml $seed^{-1}$. In each experiment, four possible treatment groups were used. Two treatments groups included seeds that were coated with the UW85 spore paste

and inoculated with either the high or low concentration of the appropriate rhizobia. Two other treatments groups included seeds that were not coated with the spore paste and also inoculated with either the high or low concentration of the appropriate rhizobia. There were also a series of checks which consisted of seed that received neither bacterial treatment.

3.1.2 *Plant Growth Conditions*

Seeds for each experiment were sown into autoclaved quartz sand (10-20 mesh) in 2 L gas-exchange pots. The gas-exchange pots were constructed from plastic sewer pipe (10-cm diameter × 25-cm depth). The gas exchange pots were surface sterilized by being soaked in a 5% sodium hypochlorite solution for 24 hours and were then rinsed thoroughly with distilled water. In experiments involving soybean and field pea, splash guards made of clear vinyl floor runner were used to prevent contamination between pots. Each cylindrical splash guard was 10 cm in height and 13.5 cm in top diameter which tapered down to 10.5 cm in bottom diameter. The splash guards were placed as a cuff around the tops of pots in order to decrease air turbulence across the surface of the sand contained in the pots as well as prevent splashing when pots were irrigated. In these experiments, each pot was also placed on a stand made out of plastic sewer pipe which was placed in a shallow plastic bowl. Liquid running through the gas exchange pots was captured in this manner and prevented from being dispersed by the air turbulence of the growth cabinet. The splash guards, stands and plastic bowls were all sterilized in the same manner as the gas exchange pots before an experiment.

All experiments were conducted in a controlled-environment cabinet (model GRV36, Econaire, Winnipeg, MB) which had all of its surfaces sterilized with a 5% sodium hypochlorite spray solution about three days before each experiment began. The cabinet was set to a 16-h light/8-h dark photoperiod. In the case of soybean, the light/dark temperatures were 25°C/19°C while for common bean these temperatures were 23°C/19°C. Two field pea experiments were conducted. One was carried out with light/dark temperatures of 22°C/17°C while another was conducted with temperatures of 17°C/12°C. The light source was provided by Cool White VHO and GRO-LUX VS VHO fluorescent lamps at a ration of 3:1 producing photosynthetic photon flux densities of 820 ± 50 μmol $m^{-2}s^{-1}$, 605 ● 50 μmol $m^{-2}s^{-1}$ and 670 ± 50 μmol $m^{-2}s^{-1}$, respectively for he soybean, field pea and common bean experiments. For the soybean and field pea experiments, plants were arranged in a completely randomized fashion within the growth cabinet and rearranged weekly to reduce any variability associated with temperature or light gradients. In the experiment involving common bean, pots containing the same treatment plus two checks were each grouped together in the cabinet to avoid cross contamination between treatments. These groups were moved to a different area of the cabinet each week and the pots within each group were randomly rearranged each week.

At planting, two seeds were placed in each pot, immediately inoculated with the appropriate rhizobia treatment and covered with quartz sand. Tools used as well as the planter's hands were all sterilized between treatments during these operations. Each treatment consisted of six pots and there were eight uninoculated check pots. Seeds were irrigated with distilled water until emergence. When both seedlings had emerged, one

was removed from each pot using sterile techniques. Emergence was recorded daily from the time of planting until plants were thinned. From then on, plants were irrigated once a day with a nutrient solution which contained 0.5 mM K_2SO_4, 0.6 mM K_2HPO_4, 0.25 mM KH_2PO_4, 0.5 mM $MgSO_4$, 0.75 mM $CaSO_4$, 37 μM Fe (as Sequestrene 330, Ciba Giegy Corp), 18 μM H_3BO_3, 4 μM $MnCl_2$, 0.4 μM $CuSO_4$, 0.35 μM $ZnSO_4$, 0.3 μM Na_2MoO_4 and 0.1 μM $CoSO_4$. The nutrient solution contained no mineral N, making plants in each experiment dependent solely on N_2 fixation as a nitrogen source. The pH of the nutrient solution was adjusted to 6.5 using H_2SO_4. Nutrient solution was stored inside the growth cabinet in a sterilized carboy during each experiment. Each treatment was irrigated with a specific individual plastic container which was sterilized once a week.

3.1.3 *Gas Exchange Measurements*

At 34 DAP for each experiment, the evolution of H_2 and CO_2 of the intact root systems of a randomly selected sample of treatment plants except controls was measured to estimate nitrogenase activity and root respiration rate. For the common bean experiment, three plants of the six in a treatment group were selected and all controls were measured. For the field pea experiments, four plants of the six in a treatment group were selected. In the soybean experiment, all six plants from each of the four treatment groups were measured. Plants were kept in the growth cabinet during the measurements. Measurements were begun four to five hours after the start of the light period and took two to three hours to complete. Root systems were isolated by capping the gas exchange pots and sealing them with Terostat IX (Teroson Gmbh, Heidelberg, Germany). This

method left shoots and roots intact and undisturbed. The sealed, gas-exchange pots were connected to a 12 channel, gas-exchange system which measures the CO_2 and H_2 evolution from the nodulated roots of up to 12 plants sequentially. The system has been previously described by Vessey (1991). Root H_2 evolution was measured in atmospheres of air and $Ar:O_2$ (79:21). When the H_2 evolution rate in air reached steady state, the input gas mixture was changed to $Ar:O_2$. The subsequent peak in H_2 evolution was considered a measurement of total nitrogenase activity (Hunt et al. 1987). The peak of H_2 evolution in $Ar:O_2$ and the steady state H_2 evolution rate in air were used to calculate the electron allocation coefficient (EAC, Edie and Philips, 1983):

$$EAC = 1 - \frac{H_2 \text{ evolution in air}}{H_2 \text{ evolution in } Ar:O_2}$$

3.1.4 *Plant Harvesting and Sample Preparation*

Plant height was measured at 34 DAP in the soybean experiment. After gas-exchange measurements were completed on an experiment, the shoots and roots from each pot were separated and the sand was washed from the roots. Total leaf area using a digital leaf area metering system was measured in the soybean experiment because pronounced visual differences were noted. All plant parts were frozen at -70°C. Nodules were picked and counted from each root system. All plant parts were then freeze-dried and weighed. After being weighed, all plant parts were finely ground in a coffee grinder. Total N of all plant material was measured by the dry combustion technique using a Leco N analyzer (model FP-428; Leco Corp., Mississauga, ON).

3.1.5 *Statistical Analyses*

The experimental design for the controlled-environment experiments was a completely randomized design consisting of five treatments with six replicates per treatment except in the case of controls where there were eight replicates. The SAS program (SAS Institute, Inc., Cary, NC) was used for statistical analysis. Analyses of variance were conducted on all data. Single degree of freedom orthogonal contrasts were used for 'a priori' comparisons. Orthogonal contrasts compared treatments with UW85 inoculation to those without and treatments with high inoculation rates of (Brady)Rhizobia to those with low inoculation rate of (Brady)Rhizobia. Means separations were performed using the LSD test at the 0.05 level for 'a posteriori' comparisons.

3.2 Field Experiments

3.2.1 *Site Selection and Preparation*

Two field experiments involving soybean (*Glycine max* L. Merr cv. Maple Ridge), common bean (*Phaseolus vulgaris* L. cv. Ex-Rico23), field pea (*Pisum sativum* L. cv. Express) and lentil (*Lens esculenta* Moench cv. Eston) were conducted in 1994 to examine the effects on plant growth and N_2 fixation of soil inoculation with an in-furrow granular formulation (MicroBio Rhizo Gen, Saskatoon, SK) of the *Bacillus cereus* strain, UW85 (here on referred to as UW85). One site was sown into a Riverdale floodplain clay at the Plant Science Research Station on the campus of the University of Manitoba located at Winnipeg (49° 53' N, 97° 10' W), Manitoba. The other site was sown into an Eigenhof clay loam at the Plant Science Research Station of the University of Manitoba located at Carman (49° 28' N, 98° W), Manitoba. Climatic information (Table 1) was derived from data supplied by Environment Canada for the Winnipeg site, and from information supplied by the University of Manitoba for the Carman site.

The Winnipeg experiment was established on land that had previously been planted to winter wheat (*Triticum aestivum* L.) in the fall of 1992 which was harvested in the summer of 1993 and then left as stubble until October of that same year. This site had not had any of the experimental species grown on it during the previous 10 years. The Carman experiment was established on land that was uncropped and mowed during the 1993 growing season. In 1992 the site had been planted to common bean.

Table 3.1: Mean temperature and precipitation levels (1994) and long-term normals (1938-1990) for the growing season months at Winnipeg and Carman, MB.

Temperature and precipitation	Month	Winnipeg[a] 1994	Winnipeg Normals[a] (1938-1990)	Carman[b] 1994	Graysville[c] Normals[a] (1938-1990)
Mean Temp (°C)	May	12.4	11.6	13.2	11.2
	June	17.9	16.9	18.1	16.5
	July	18.2	19.8	18.7	19.2
	August	16.6	18.3	17.4	17.9
	September	14.2	12.4	14.7	11.9
	October	8.1	5.7	7.7	5.5
Precip. (mm)	May	69.7	59.8	41.0	52.1
	June	80.0	83.8	27.0	76.8
	July	148.3	72.0	66.0	63.1
	August	123.0	75.3	70.0	61.2
	September	69.6	51.3	63.0	53.7
	October	87.6	29.5	133.0	30

[a] Climatic information for Winnipeg derived from Environment Canada data.
[b] Climatic information for Carman derived from University of Manitoba data.
[c] Graysville was the closest weather data gathering site to the Carman experimental location (Approximately 10 miles east of Carman).

In October of 1993 soil from both sites was tested (Norwest Labs, Winnipeg, MB). Each site was divided into four quadrants and four samples from two depths (0 to 15 cm and 15 to 60 cm) were taken from each quadrant. Samples were then bulked for each depth within each quadrant. Average macronutrient levels at the Winnipeg site to a 60 cm depth were as follows: N - 58 kg ha^{-1}, P_2O_5 - 52 kg ha^{-1}, K - 868 kg ha^{-1}, S - 34 kg ha^{-1}. Soil pH in the 0 to 15 cm layer was 7.5. Average macronutrient levels at the Carman site to a 60 cm depth were as follows: N - 66 kg ha^{-1}, P_2O_5 - 48 kg ha^{-1}, K - 854 kg ha^{-1}, S - 34 kg ha^{-1}. Soil pH in the 0 to 15 cm soil layer was 7.2. In 1994 triple superphosphate fertilizer (0-46-0) was broadcast applied at 40 kg P_2O_5 ha^{-1} to the Winnipeg site on May 6 and the Carman site on May 9 and then incorporated to an approximate 10 cm depth at both sites using a vibrashank field cultivator and diamond tooth harrows. The soil at the Carman site was also packed using a roller-packer unit at this time.

A combination of herbicides and tillage was used for pre-emergent weed control at both experimental sites. Trifluralin as Treflan QR5 (1.06 kg a.i. ha^{-1}) was broadcast applied onto the Carman site on October 18, 1993 and then incorporated to an approximate 10 cm depth using a vibrashank field cultivator. A second tillage pass to the same depth and using the same equipment but a right angles to the first was done four days later. At the Winnipeg site, Trifluralin as Treflan QR5 (1.70 kg a.i. ha^{-1}) was broadcast applied on October 18, 1993 and then incorporated to an approximate 10 cm depth using a tandem disc. A second tillage pass was made at this site in the spring of 1994 when fertilizer was being incorporated. At one week before planting, both experimental sites were sprayed with glyphosate with the Carman site receiving 3.32 kg

a.i. ha^{-1} and the Winnipeg site receiving 1.19 kg a.i. ha^{-1}.

3.2.2 *Site Design, Seeding and Maintenance*

Both field experiments were arranged as randomized complete block designs with four blocks. Individual plots (4 m X 6 m) were spaced 1 m apart within blocks. Blocks were separated by alleys which measured 5 m in Winnipeg and 8 m in Carman. With each of the four host species, four different inoculation treatments were used. These included no inoculation (-R-B), in-furrow soil inoculation with a granular formulation of UW85 (-R+B), seed inoculation with an appropriate *Rhizobium* or *Bradyrhizobium* peat-based inoculant (+R-B) and a combination of in-furrow soil inoculation with the granular inoculant and seed inoculation with the appropriate peat-based inoculant (+R+B).

Peat-based *Rhizobium* and *Bradyrhizobium* inoculants used in these experiments were MBR Self-Stick sterile peat products (MicroBio RhizoGen Corp., Saskatoon, SK). All of the inoculants were applied at the manufacturer's recommended rate of 3.3 kg per 820 kg of seed. A 10% solution of sucrose was added during the preparation of seed to enhance the adhesion of the inoculant. Seed was inoculated with the appropriate peat-based inoculant the evening before the day it was to be seeded and stored in a cooler at 5°C until sowing.

On the day of seeding the concentration of UW85 spores on the granular inoculant was quantified using the following procedure. Under sterile conditions, two 10 g samples of the granules were obtained from the supply to be used for that day's seeding. Each of the samples were then added to 90 mls of sterile buffered solution. This solution

contained 1.0 g peptone, 0.34 g KH_2PO_4 and 0.21 g K_2HPO_4 which was mixed in 1 L of water and was autoclaved. The mixtures of granules and dilutent were then manually shaken for three minutes and left to stand for 30 minutes. Each solution was then added to sterile plastic jars which were fitted to an electric blender. The solutions were blended for three minutes at low speed. A 1 ml sample of each solution was serially diluted using sterile buffered dilutent. The dilutions were plated on sterile RBM media (MicroBio RhizoGen Corp. Saskatoon, SK) which contained 3.0 g Universal Polypeptone, 0.6 g Difco Yeast Extract, 2.6 g sucrose, 1.0 ml glycerol, 0.60 g KNO_3, 0.10 g NaCl, 0.50 g K_2HPO_4, 0.50 g $MgCO_3$ and 10.0 g of agar mixed in 1 L of water. Plates were incubated at 28°C for 24 hours and then counted. The UW85 granular inoculant was found to have a concentration of 1.0×10^8 to 2.0×10^8 CFU g^{-1}.

All four grain legume species were sown at the Carman site on May 16, 1994. Previously, all seed stocks had been germination tested and 1000 seed weights had been determined. Seeding was done using a Fabro offset disc opener press drill (Fabro Manufacturing Ltd., Swift Current, SK) equipped with a cone and splitter attachment. The drill was 2 m wide and could seed twelve rows in one pass. The drill made two passes through each plot and the number of rows seeded was reduced from twelve to three rows per pass by switching from a twelve row to a six row splitter and bagging three of the rows. Amounts of seed and inoculant used with this modified set up were adjusted accordingly to maintain correct seeding and inoculation rates. For soybean and common bean, six rows 6 m in length and spaced 61 cm apart were sown in each plot. For field pea and lentil, 24 rows 6 m in length and spaced 16 cm apart were sown in each

plot. Soybean and lentil were sown at a depth of 2.5 cm while common bean and field pea were sown at depth of 5.0 cm. The seeding rates used at the Carman site were as follows: soybean - 65 kg of viable seed ha^{-1} or 26 viable seeds m^{-1} of seed row, common bean - 22.5 kg of viable seed ha^{-1} or 7 viable seeds m^{-1} of seedrow, field pea - 170 kg of viable seed ha^{-1} or 20 viable seeds m^{-1} of seedrow, lentil - 35 kg of viable seed ha^{-1} or 18 viable seeds m^{-1} of seedrow. The granular UW85 inoculant was applied with seed of each crop at a rate of 1 g m of $seedrow^{-1}$ or 15 kg ha^{-1} in the case of soybean and common bean and 60 kg ha^{-1} in the case of field pea and lentil. When needed, the granular inoculant was added first to the cone using a calibrated plastic scoop and seed was then added. Regardless of the crop, treatments were always planted in the following order: 1) uninoculated seed 2) UW85 treated seed 3) (Brady)Rhizobia treated seed 4) UW85 and (Brady)Rhizobia treated seed. Before seeding the third treatment, the cone and spinner apparatus of the drill was wiped down with a 70% solution of ethanol and allowed to dry. The seeder was equipped with clear, static-free seed tubes which were shaken after each treatment was seeded.

At the Winnipeg site, the soybean and lentil were sown on June 1, 1994 to an approximate depth of 3.0 cm. Common bean and field pea were sown on June 8, 1994 to an approximate depth of 4.0 cm. The same equipment and methodology outlined for the Carman seeding date was used on these seeding dates. The seeding rates used at the Winnipeg site were as follows: soybean - 130 kg of viable seed ha^{-1} or 52 viable seeds m^{-1} of seed row, common bean - 45 kg of viable seed ha^{-1} or 14 viable seeds m^{-1} of seedrow, field pea - 170 kg of viable seed ha^{-1} or 20 viable seeds m^{-1} of seedrow, lentil -

35 kg of viable seed ha^{-1} or 18 viable seeds m^{-1} of seedrow.

A combination of herbicides, hand-weeding and tillage was used for post-emergent weed control at both experimental sites. Sethoxydim (0.496 kg a.i. ha^{-1}) was used at Carman on June 17, 1994 and at Winnipeg on June 22, 1994. Imazethapyr (0.050 kg a.i. ha^{-1}) was used at the Carman site only July 7, 1994 for all crops except lentil. Redbacked cutworms (*Euxoa ochrogaster* Guenee) were present at the Carman site and were controlled with an application of chlorpyrifos (0.575 kg a.i. ha^{-1}) on June 21, 1994. Lentil plots at the Winnipeg site were sprayed with chlorothalonil (1.00 kg a.i. ha^{-1}) on July 12, 1994.

3.2.3 *Plant Harvesting and Tissue Analysis*

At both sites, plant stand counts were carried out for all crops at approximately 60 days after planting (DAP). In the case of soybean and common bean, three samples of 3 m of row each were counted. Plots were roughly divided into two halves lengthwise with three of eight possible 3 m segments being randomly selected for counting. Because of the occurrence of insect damage and areas of poor emergence, row samples with canopy breaks were not used for any sampling. Outside rows were also not used for any sampling. Counts were begun approximately 50 cm in from the end of a row. For field pea and lentil, two 1 m^2 samples were randomly selected from each plot and counted. Square metre samples were selected so that the same number and length of rows were used for all sampling.

The dry matter harvests of soybean and common bean grown at Winnipeg and

Carman were conducted when plants were at early pod-filling (soybean - growth stage R4, common bean - growth stage R7, approximately 60 DAP), physiological maturity (soybean - growth stage R7, common bean - growth stage R8, approximately 90 DAP) and harvest maturity (soybean - growth stage R8, common bean - growth stage R9, approximately 120 DAP). In the first and second harvests, two samples of 3 m each were taken from each plot in a similar manner as outlined previously for stand counts with one sample selected from each half of the plot. In the final harvests, 2.5 m samples of row were collected. All samples were frozen after harvest until further processing could be done.

Frozen samples from the first harvests were oven dried at 70°C until daily checks indicated no further decreases in weight. At that point, all samples were weighed and ground in a Wiley mill using a 2.0 mm screen. N concentration of the ground material was measured by a combustion technique using a Leco N analyzer (Model FP 428; Leco Corp., Mississauga, ON).

Pods were separated from the shoots of plants for each of the frozen samples from the second and final harvests. Pod and plant numbers per sample were determined at this time. Shoots and pods were then oven dried at 70°C as previously described for the first harvest. Dried shoots and pod were then weighed. Shoots were ground in a Wiley mill using a 2.0 mm screen. Seed was harvested from pods using a stationary belt thresher and the harvested samples were then weighed. The number of seeds per harvested sample was determined by weighing 250 seeds from each sample. A selection of harvested seed samples were then oven dried at 70°C once again and weighed. Seed was then ground in

an Udy cyclone mill using a 0.5 mm sieve. When seed harvesting had been done, the empty pods generated from each sample were collected, oven-dried at 70°C and ground in a Wiley mill using a 2.0 mm screen. The N concentration of all the ground plant tissue samples were determined by a combustion technique using a Leco N analyzer.

The first harvests of field pea and lentil were conducted at the Winnipeg and Carman sites when field pea and lentil plants were at early pod-filling (approximately 60 DAP). Two 1 m^2 samples were randomly selected from each plot and harvested. Square metre samples were selected so that the same number and length of rows were used for all sampling. All samples were frozen until they were oven-dried at 70°C as previously described. Samples were then weighed, ground and measured for N concentration as previously described for other harvests.

At the time of the first harvest of field pea and lentil at both locations, disease symptoms were noted on plants which bordered plots of both species. Symptoms included stunted growth accompanied with wilting and browning of leaves. In the period between the first and second harvest, the symptoms progressed from the borders inwards until all plots were completely brown and wilted. Stems were often pinched at or just below the soil surface and pinkish and greyish mold growth was often present. As the disease advanced, plants could be detached easily at the soil surface. Visual assessments suggested that the plots had succumbed to Fusarium wilt cause by *Fusarium* sp. based on symptom descriptions by Haglund (1984). At that time, it was decided to terminate field pea and lentil plots such that data for these crops exists for only the early pod-filling stage. No differences in disease severity were noted among the different treatments for

either field pea or lentil.

3.2.4 *Nitrogenase Activity Measurements*

Just prior to the first harvest of plots of each crop at both experimental sites (approximately 60 DAP), randomly chosen plants from each plot were analyzed for nitrogenase activity by an acetylene reduction assay. Sampling in the field was conducted between approximately 10 a.m. and 2 p.m. Two plants from each plot were cut off at the base of the stem and soil cores of the upper root and root crown were extracted from the soil. Each core was 6.4 cm in diameter and 15 cm in depth. Each core was placed in a 1 L mason jar and sealed. Approximately 10% of the air volume was removed from each jar with a syringe and replace with acetylene. After 30 minutes of incubation, a 10 cm^3 gas sample was removed from each jar and the concentration of ethylene was later determined by flame ionization detection on a Carle (model 311) gas chromatograph (1.8 m by 1.6 mm (ID) column with Porapak T (50/80) packing at 80°C).

3.2.5 *Statistical Analyses*

Treatments were arranged in a randomized complete block design with four replicates for field experiments at the Winnipeg and Carman sites in 1994. The SAS program (SAS Institute, Inc., Cary, NC) was used for statistical analysis. Analyses of variance were conducted on data and single degree of freedom orthogonal contrasts were used for 'a priori' comparisons. Orthogonal contrasts compared treatments with UW85 inoculation to those without and treatments with (Brady)Rhizobia inoculation to those

uninoculated. Means separations were performed using the LSD test at the 0.05 level for 'a posteriori' comparisons.

4.0 Results

4.1 Controlled-Environment Studies - Soybean

4.1.1 *Seedling Emergence*

At 10 days after planting (DAP), 100% of seeds used in this experiment had emerged. No visual treatment differences in seedling appearance were observed.

4.1.2 *Dry Matter Yield*

Whole plant dry matter yield (DM) (Table 4.1) was 112 % greater for plants with higher inoculation rates of *B. japonicum* (HR+B and HR-B) as compared to those with lower inoculation rates (LR+B and LR-B). As well, whole plant DM was 21% greater among *B. japonicum* inoculated plants that also received *B. cereus* strain (HR+B and LR+B) versus those that did not receive *B. cereus* (HR-B and+LR-B). A 24% increase in whole plant DM was found for HR+B treated plants versus HR-B treated plants.

Stem and leaf DM (Table 4.1) was 142% greater for plants with higher inoculation rates of *B. japonicum* and 20 % greater among *B. japonicum* inoculated plants that also received *B. cereus*. A 27% increase was found for HR+B treated plants as compared to HR-B treated plants.

Root DM (Table 4.1) was 49 % greater for plants with higher inoculation rates of *B. japonicum*. Among *B. japonicum* inoculated plants, those that also received *B. cereus* had 22 % greater root DM. Root DM was 18 % greater for HR+B treated plants compared to the HR-B treated plants, and 22% greater for LR+B treated

Table 4.1: DM (mg), % N and N content (mg N) of 34 day old soybean uninoculated (C), or inoculated with higher (HR) or lower (LR) rates of *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
DM (mg)	Whole Plant	2960 ± 190	2380 ± 90	1330 ± 110	1180 ± 80	1230 ± 50	Rhiz**[c] Bac*[d]	340
	Stem and Leaves	2000 ± 150	1580 ± 60	760 ± 80	720 ± 70	620 ± 40	Rhiz** Bac*	250
	Root	720 ± 50	610 ± 30	500 ± 40	390 ± 30	610 ± 20	Rhiz** Bac*	110
	Nodules	236 ± 23	188 ± 9	73 ± 10	69 ± 8	N/A	Rhiz** Bac*	44
%N	Whole Plant	2.74 ± 0.06	2.97 ± 0.06	2.01 ± 0.15	2.06 ± 0.13	1.10 ± 0.00	Rhiz**	0.26
	Stem and Leaves	2.78 ± 0.06	3.05 ± 0.08	2.03 ± 0.15	1.99 ± 0.16	1.06 ± 0.01	Rhiz**	0.28
	Root	1.65 ± 0.07	1.89 ± 0.11	1.39 ± 0.05	1.49 ± 0.03	1.16 ± 0.05	Rhiz** Bac*	0.20
	Nodules	5.71 ± 0.06	5.84 ± 0.11	5.69 ± 0.63	5.97 ± 0.16	N/A	NS	NS
N(mg)	Whole Plant	81.2 ± 5.9	70.7 ± 3.2	26.8 ± 3.0	24.4 ± 2.5	13.6 ±0.8	Rhiz** Bac*	10.1
	Stem and Leaves	56.0 ± 4.8	48.3 ± 2.6	15.5 ± 2.0	14.5± 2.0	6.6 ± 0.3	Rhiz**	8.0
	Root	11.8 ± 0.8	11.5 ± 0.8	6.8 ± 0.4	5.9 ± 0.4	7.0 ± 0.4	Rhiz**	1.8
	Nodules	13.4 ± 1.3	11.0 ± 0.4	4.4 ± 0.8	4.1 ± 0.5	N/A	Rhiz** Bac*	2.6

[a] Measures for all plant part(s) expressed as treatment group mean ± SEM.

[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

[c] Indicates contrast statements that were significant at P=0.01.

[d] Indicates contrast statements that were significant at P=0.05.

plants compared to the LR-B treated plants. It should be noted that control plants had root DM that did not differ from HR-B treated plants.

Nodule DM (Table 4.1) was 199% greater for plants with higher inoculation rates of *B. japonicum*. A 26% increase in nodule DM was found for HR+B treated plants compared to HR-B treated plants.

4.1.3 *Nitrogen Concentration*

Whole plant N concentration (Table 4.1) was 40% greater for plants with higher inoculation rates of *B. japonicum*. *B. cereus* inoculation had no effect on whole plant N concentration while *B. japonicum* inoculation resulted in greater whole plant N concentrations than were found for control plants.

Stem and leaf N concentration (Table 4.1) was 45% greater for plants with higher inoculation rates of *B. japonicum*. *B. cereus* inoculation had no effect on stem and leaf N concentration while *B. japonicum* inoculation resulted in greater stem and leaf N concentrations than were found for control plants.

Root N concentration (Table 4.1) was 23% greater for plants with higher inoculation rates of *B. japonicum* and 11% greater among *B. japonicum* inoculated plants which had not received *B. cereus*. HR-B treated plants had root N concentrations that were 15%, 27% and 36% greater than HR+B, LR-B and LR+B treated plants, respectively. *B. japonicum* inoculation resulted in greater root N concentrations than were found for control plants. No differences in nodule N concentration (Table 4.1) were found.

4.1.4 *Nitrogen Content*

Whole plant N content (Table 4.1) was 197% greater for plants with higher inoculation rates of *B. japonicum* and 14% greater among plants inoculated with *B. japonicum* that also received *B. cereus*. HR+B treated plants had 15% greater whole plant N content than HR-B treated plants and *B. japonicum* inoculation resulted in greater whole plant N contents than were found for control plants.

Stem and leaf N content (Table 4.1) was 248% greater for plants with higher inoculation rates of *B. japonicum*. Root N content (Table 4.1)was 83% greater for plants with higher inoculation rates of *B. japonicum*. *B. cereus* inoculation had no effect of stem and leaf or root N content but *B. japonicum* inoculation resulted in greater stem and leaf and root N contents than were found for control plants.

Nodule N content (Table 4.1) was 187% higher for plants with higher rates of *B. japonicum* inoculation. A 22% increase in nodule N content was found for HR+B treated plants compared to HR-B treated plants.

4.1.5 *Leaf Area and Plant Height Measurements*

Total leaf area (Table 4.2) was 132% greater for plants with higher inoculation rates of *B. japonicum* and 15% greater among *B. japonicum* inoculated plants that also received *B. cereus*. HR+B treated plants had 19% greater total leaf area than HR-B treated plants.

Plant height (Table 4.2) was 22% greater for plants with higher *B. japonicum* inoculation rates and 12% greater among *B. japonicum* inoculated plants which also

Table 4.2: Total leaf area (cm^2) and plant height (cm) of 34 day old soybean uninoculated (C), or inoculated with high (HR) or low (LR) rates of *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
Leaf Area (cm^2)	244 ± 18	205 ± 7	100 ± 9	94 ± 9	71 ± 4	Rhiz**[c] Bac*[d]	29
Plant Height (cm)	14.6 ± 1.0	12.3 ± 0.3	11.4 ± 0.8	10.8 ± 1.0	11.8 ± 0.2	Rhiz* Bac*	2.0

[a] Measures for all parameters expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

received *B. cereus*. HR+B treated plants had plant heights that were 19% greater than HR-B treated plants.

4.1.6 *Root Nodule and N_2 Fixation Measurements*

Root nodule number (Table 4.3) was 103% greater for plants with higher inoculation rates of *B. japonicum* and 19% greater among *B. japonicum* inoculated plants which also received *B. cereus*. A 27% increase in root nodule number for HR+B treated plants versus HR-B treated plants was found.

Individual nodule DM (Table 4.3) was 35% greater for plants with higher inoculation rates of *B. japonicum.* No differences among treatments in nodule number g^{-1} root DM were found.

Plants inoculated with lower rates of *B. japonicum* had 24% greater specific nitrogenase activity (Table 4.3) than those inoculated with higher rates of *B. japonicum.* LR+B treated plants had 34 % greater specific nitrogenase activity levels than HR+B treated plants while LR-B treated plants had 30% greater specific nitrogenase activity levels than HR+B treated plants. No differences in specific nitrogenase activity were found between LR +B, LR-B and HR-B treated plants. No differences among treatments in nitrogen fixation efficiencies (Table 4.3) were found.

Table 4.3: Nodule number, individual nodule DM, specific root nodulation , specific nitrogenase activity and nitrogen fixation efficiency of 34 day old soybean uninoculated (C), or inoculated with high (HR) or low (LR) rates of *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
Nodule Number Plant^{-1}	70± 7	55 ± 5	31 ± 8	30 ± 4	0	Rhiz**[c] Bac*[d]	16
Individual Nodule DM (mg)	3.4 ± 0.2	3.6 ± 0.3	2.7 ± 0.5	2.4 ± 0.5	N/A	Rhiz*	1.1
Specific Root Nodulation[e]	99 ± 10	91 ± 9	66 ± 18	78 ± 10	N/A	NS	NS
Specific Nitrogenase Activity[f]	226.5 ± 15.0	254.9 ± 15.0	304.4 ± 21.7	294.7 ± 18.0	N/A	Rhiz*	53.8
Nitrogen Fixation Efficiency[g]	0.35 ± 0.01	0.38 ● 0.01	0.37 ± 0.02	0.36 ● 0.01	N/A	NS	NS

[a] Measures for all parameters expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.
[e] Specific Root Nodulation = nodule number g^{-1} root DM.
[f] Specific Nitrogenase Activity = μmol H_2 evolved in Ar:O_2 g^{-1} nodule DM $hour^{-1}$.
[g] N-fix Efficiency = total plant N (mg) $nodule^{-1}$ DM (mg).

4.2 Controlled-Environment Studies - Common Bean

4.2.1 *Seedling Emergence*

At 10 days after planting (DAP), 77% of control seeds (C), 75% of seeds inoculated with lower rates of *R. etli* alone (LR-B) and 100% of seeds inoculated with higher rates of *R. etli* alone (HR-B) had emerged. At the same time, only 58% of seeds inoculated with lower rates of *R. etli* and *B. cereus* (LR+B) and 54% of seeds inoculated with higher rates of *R. etli* and *B. cereus* (HR+B) had emerged. Seedlings inoculated with *B. cereus* were noted as having pruned and blackened roots and brown cotyledon spots (Figure 1).

4.2.2 *Dry Matter Yield*

Whole plant dry matter yield (DM) (Table 4.4) did not differ among plants which had received *R. etli* inoculation. *R. etli* inoculation resulted in greater whole plant DM than was found for control plants. As well, stem and leaf, root and nodule DM (Table 4.4) did not differ among plants which had received *R. etli* inoculation. As expected, *R. etli* inoculation resulted in greater plant part DM than were found for control plants.

4.2.3 *Nitrogen Concentration*

No differences among treatments were found in the N concentrations of whole plants, stems and leaves, roots or nodules (Table 4.4).

Figure 4.1: Healthy common bean seedling (first on left) and *B. cereus* UW85 inoculated common bean seedlings (right) which exhibit pruned and blackened roots and brown cotyledon spots.

4.2.4 *Nitrogen Content*

No differences among treatments which included *R. etli* inoculation were found for whole plant, stem and leaf, root or nodule N content (Table 4.4). *R. etli* inoculation resulted in greater whole plant and plant part N contents than were found for control plants.

4.2.5 *Root Nodule and N_2 Fixation Measurements*

Root nodule numbers (Table 4.5) did not differ among treatments which included *R. etli* inoculation. *R. etli* inoculation resulted in greater root nodule numbers than were found for control plants. As well, no differences among treatments in individual nodule DM (Table 4.5) were found. HR+B, HR-B and LR+B treated plants had 111%, 103% and 101% greater specific root nodulation levels, respectively, than control plants (Table 4.5). No differences among treatments for specific nitrogenase activity or nitrogen fixation efficiency (Table 4.5) were found.

Table 4.4: DM (mg), % N and N content (mg N) of 34 day old common bean uninoculated (C), or inoculated with high (HR) or low (LR) rates of *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
DM (mg)	Whole Plant	2756 ± 315	2591 ± 151	2365 ± 133	2677 ± 204	1348 ± 253	NS	748
	Stem and Leaves	1644 ± 176	1483 ± 116	1434 ± 80	1585 ± 103	663 ± 150	NS	440
	Root	781 ± 120	801 ± 71	657 ± 54	809 ± 117	550 ± 104	NS	317
	Nodules	331 ± 45	307 ± 27	274 ± 16	283 ± 13	135 ± 3	NS	99
%N	Whole Plant	3.55 ± 0.13	3.54 ± 0.09	3.93 ± 0.06	3.70 ± 0.10	3.06 ± 0.28	NS	0.70
	Stem and Leaves	4.07 ± 0.06	4.10 ± 0.20	4.53 ± 0.10	4.29 ± 0.12	3.63 ± 0.43	NS	1.04
	Root	2.03 ± 0.20	2.16 ± 0.14	2.23 ± 0.09	2.17 ± 0.14	1.85 ± 0.10	NS	0.40
	Nodules	4.79 ± 0.08	4.77 ± 0.08	4.91 ± 0.06	4.88 ± 0.09	3.78 ± 0.66	NS	1.60
N(mg)	Whole Plant	106.9 ± 9.0	92.0 ± 6.3	93.1 ± 6.0	98.3 ± 5.3	46.4 ± 10.7	NS	29.3
	Stem and Leaves	67.2 ± 7.7	60.5 ± 4.3	65.0 ± 3.8	67.6 ± 3.6	30.0 ± 8.0	NS	21.6
	Root	15.1 ± 2.1	16.9 ± 0.8	14.7 ± 1.4	16.9 ± 1.7	9.5 ± 1.5	NS	4.9
	Nodules	17.4 ± 1.8	14.7 ± 1.4	13.4 ± 0.8	13.8 ± 0.5	6.8 ± 1.7	NS	4.7

[a] Measures for all plant part(s) expressed as treatment group mean ● SEM.

[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

[c] Indicates contrast statements that were significant at P=0.01.

[d] Indicates contrast statements that were significant at P=0.05.

Table 4.5: Nodule number, individual nodule DM, specific root nodulation, specific nitrogenase activity and nitrogen fixation efficiency of 34 day old common bean uninoculated (C), or inoculated with high (HR) or low (LR) rates of *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
Nodule Number Plant^{-1}	619 ● 104	676 ± 102	551 ± 75	526 ± 43	217 ± 66	NS	240
Individual Nodule DM (mg)	0.60 ± 0.12	0.50 ± 0.03	0.54 ± 0.06	0.55 ± 0.03	0.51 ± 0.12	NS	0.31
Specific Root Nodulation[c]	887 ± 189	854 ± 137	846 ± 110	692 ± 78	421 ± 100	NS	376
Specific Nitrogenase Activity[d]	180.6 ± 12.4	171.0 ± 12.3	212.5 ± 26.7	189.0 ± 23.1	213.1 ± 14.7	NS	58.3
Nitrogen Fixation Efficiency[e]	0.30 ± 0.02	0.30 ± 0.01	0.34 ± 0.01	0.35 ± 0.02	0.23 ± 0.04	NS	0.11

[a] Measures for all parameters expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Specific Root Nodulation = nodule number g^{-1} root DM.
[d] Specific Nitrogenase Activity = μmol H_2 evolved in Ar:O_2 g^{-1} nodule DM hour^{-1}.
[e] N-fix Efficiency = total plant N (mg) nodule^{-1} DM (mg).

4.3 Controlled-Environment Studies - Field Pea (22°C day/17°C night temp regime)

4.3.1 *Seedling Emergence*

At 10 days after planting (DAP) 100% uninoculated control seeds (C), seeds inoculated with lower rates of *R. leguminosarum* bv. *viceae* alone (LR-B), seeds inoculated with higher rates of *R. leguminosarum* bv. *viceae* alone (HR-B) and seeds inoculated with lower rates of *R. leguminosarum* bv. *viceae* and *B. cereus* (LR+B) had emerged. At the same time, 92% of seeds inoculated with higher rates of *R. leguminosarum* bv. *viceae* and *B. cereus* (HR+B) had emerged.

4.3.2 *Dry Matter Yield*

Whole plant dry matter yield (DM) (Table 4.6) was 22% greater for LR-B treated plants compared to LR+B treated plants. Also, LR-B treated plants had 23% greater whole plant DM compared to HR-B treated plants. All treatments which included inoculation with *R. leguminosarum* bv. *viceae* resulted in greater whole plant DM than was found for control plants.

Stem and leaf DM (Table 4.6) did not differ among treatments. Root DM (Table 4.6) was 21% greater for plants treated with lower inoculation rates of *R. leguminosarum* bv. *viceae* (LR+B and LR-B) compared to those treated with higher inoculation rates (HR+B and HR-B). As well, root DM was 17% greater for *R. leguminosarum* bv. *viceae* inoculated plants which did not receive *B. cereus* (HR-B and LR-B) compared to those which did receive *B. cereus* (HR+B and LR+B). LR-B treated plants were found to have

Table 4.6: DM (mg), %N and N content (mg N) of 34 day old field pea uninoculated (C), or inoculated with high (HR) or low (LR) rates of *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85 and grown in a 22°C day/17°C night temperature regime.

Parameter	Plant Part(s)[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
DM (mg)	Whole Plant	2821 ±167	2541 ±154	2577 ± 54	3137 ± 189	536 ± 54	Bac**[c]	386
	Stem and Leaves	1856 ● 142	1591 ± 106	1612 ± 53	1850 ± 147	204 ± 18	NS	298
	Root	806 ± 60	797 ± 68	824 ± 85	1117 ± 77	332 ± 40	Rhiz*[d] Bac**	194
	Nodules	159 ± 8	154 ± 7	142 ± 8	171 ± 5	N/A	Bac**	21
%N	Whole Plant	4.37 ± 0.10	4.40 ± 0.10	4.40 ± 0.16	3.98 ± 0.11	1.52 ± 0.07	Bac*	0.32
	Stem and Leaves	4.44 ± 0.14	4.66 ± 0.12	4.78 ± 0.09	4.43 ± 0.12	1.26 ± 0.30	Bac*	0.30
	Root	3.34 ± 0.17	3.09 ± 0.10	2.96 ± 0.26	2.66 ± 0.22	1.70 ± 0.12	NS	0.52
	Nodules	9.05 ± 0.11	8.59 ± 0.24	8.85 ± 0.19	8.18 ± 0.38	N/A	Bac*	0.74
N(mg)	Whole Plant	123.8 ± 9.0	111.3 ± 5.2	113.0 ± 2.5	125.0 ± 9.1	8.1 ± 0.8	NS	17.7
	Stem and Leaves	82.8 ± 7.0	73.6 ± 3.7	77.0 ± 2.5	81.8 ± 6.6	2.6 ± 0.2	NS	13.3
	Root	26.7 ± 1.6	24.5 ● 1.8	23.4 ± 1.2	29.2 ± 2.0	5.6 ● 0.6	Bac*	4.3
	Nodules	14.3 ● 0.6	13.1 ± 0.4	12.6 ● 0.8	14.0 ± 0.9	N/A	NS	NS

[a] Measures for all plant part(s) expressed as treatment group mean ± SEM.

[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

[c] Indicates contrast statements that were significant at P=0.01.

[d] Indicates contrast statements that were significant at P=0.05.

36%, 40% and 39% greater root DM than LR+B, HR-B and HR+B treated plants, respectively. For both stems and leaves and roots, *R. leguminosarum* bv. *viceae* inoculation resulted in greater DM than were found for control plants. LR-B treated plants had 20% greater nodule DM than LR+B treated plants.

4.3.3 *Nitrogen Concentration*

Whole plant N concentration (Table 4.6) of LR+B and HR-B treated plants was 11% greater than LR-B treated plants. As well, HR+B treated plants had 10% greater whole plant N concentration than LR-B treated plants. Stem and leaf N concentration (Table 4.6) was 8% greater for LR+B treated plants compared to LR-B and HR+B treated plants. Root N concentration (Table 4.6) was 26% greater for HR+B treated plants compared to LR-B treated plants. For whole plants, stems and leaves and roots, *R. leguminosarum* bv. *viceae* inoculation resulted in greater N concentrations than were found for control plants. Nodule N concentration (Table 4.6) was 7% greater for *R. leguminosarum* bv. *viceae* inoculated plants which received *B. cereus*. HR+B treated plants had 11% greater nodule N concentration than LR-B treated plants.

4.3.4 *Nitrogen Content*

Whole plant and stem and leaf N content (Table 4.6) did not differ among treatments which included *R. leguminosarum* bv. *viceae* inoculation. However, *R. leguminosarum* bv. *viceae* inoculation resulted in greater whole plant and stem and leaf N contents than were found for control plants. Root N content (Table 4.6) of LR-B treated

plants was 20% and 19% greater than LR+B and HR-B treated plants, respectively. Nodule N content (Table 4.6) did not differ among treatments.

4.3.5 *Root Nodule and N_2 Fixation Measurements*

No differences among treatments were found for root nodule number, individual nodule DM, specific root nodulation, specific nitrogenase activity or nitrogen fixation efficiency (Table 4.7).

Table 4.7: Nodule number, individual nodule DM, specific root nodulation, specific nitrogenase activity and nitrogen fixation efficiency of 34 day old field pea uninoculated (C), or inoculated with high (HR) or low (LR) rates of *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85 and grown in a 22°C day/17°C night temperature regime.

Parameter[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
Nodule Number Plant^{-1}	325 ± 57	288 ± 55	252 ± 23	264 ± 26	0	NS	108
Individual Nodule DM (mg)	0.58 ± 0.12	0.59 ± 0.07	0.58 ± 0.06	0.68 ± 0.06	N/A	NS	NS
Specific Root Nodulation[c]	409 ± 77	369 ± 70	336 ± 62	238 ± 20	N/A	NS	NS
Specific Nitrogenase Activity[d]	164.8 ± 9.5	291.5 ± 56.5	246.2 ± 63.4	295.4 ± 13.1	N/A	NS	NS
Nitrogen Fixation Efficiency[e]	0.78 ± 0.05	0.73 ±0.03	0.81 ± 0.03	0.73 ± 0.04	N/A	NS	NS

[a] Measures for all parameters expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Specific Root Nodulation = nodule number g^{-1} root DM.
[d] Specific Nitrogenase Activity = μmol H_2 evolved in $Ar{:}O_2$ g^{-1} nodule DM $hour^{-1}$.
[e] N-fix Efficiency = total plant N (mg) $nodule^{-1}$ DM (mg).

4.4 Controlled-Environment Studies - Field Pea (17°C day/12°C night temp regime)

4.4.1 *Seedling Emergence*

At 10 days after planting (DAP), 100% of seeds inoculated with lower rates of *R. leguminosarum* bv. *viceae* alone (LR-B), seeds inoculated with higher rates of *R. leguminosarum* bv. *viceae* alone (HR-B) and seeds inoculated with lower rates of *R. leguminosarum* bv. *viceae* and *B. cereus* had emerged. At this time, 92% of seeds inoculated with higher rates of *R. leguminosarum* bv. *viceae* and *B. cereus* (HR+B) and uninoculated control seeds (C) had emerged.

4.4.2 *Dry Matter Yield*

Whole plant and stem and leaf dry matter yield (DM) (Table 4.8) did not differ among treatments which included inoculation with *R. leguminosarum* bv. *viceae*. However, inoculation with *R. leguminosarum* bv. *viceae* resulted in greater whole plant and stem and leaf DM than were found for control plants. No differences among treatments in root or nodule DM (Table 4.8) were found.

4.4.3 *Nitrogen Concentration*

Whole plant, stem and leaf and root N concentrations (Table 4.8) did not differ among treatments which included *R. leguminosarum* bv. *viceae* inoculation. *R. leguminosarum* bv. *viceae* inoculation did result in greater whole plant and plant part N concentrations than were found for control plants. No differences among treatments for

Table 4.8: DM (mg), %N and N content (mg N) of 34 day old field pea uninoculated (C), or inoculated with high (HR) or low (LR) rates of *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85 and grown in a 17°C day/12°C night temperature regime.

Parameter	Plant Part(s)[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
DM (mg)	Whole Plant	1372 ± 55	1448 ± 54	1531 ± 180	1291 ± 55	780 ± 69	NS	276
	Stem and Leaves	843 ± 54	932 ± 46	1022 ± 150	818 ± 17	266 ± 16	NS	211
	Root	446 ± 34	434 ± 35	428 ± 40	392 ± 47	514 ± 64	NS	137
	Nodules	82 ± 5	82 ± 4	81 ± 5	81 ± 4	N/A	NS	NS
%N	Whole Plant	4.21 ± 0.14	4.48 ± 0.08	4.35 ± 0.14	4.50 ± 0.17	1.27 ± 0.07	NS	0.38
	Stem and Leaves	4.51 ± 0.19	4.73 ± 0.09	4.66 ± 0.12	4.79 ± 0.12	1.13 ± 0.02	NS	0.35
	Root	2.75 ± 0.09	3.10 ± 0.15	2.82 ± 0.25	3.01 ± 0.20	1.29 ± 0.15	NS	0.50
	Nodules	8.85 ± 0.28	9.10 ± 0.13	8.86 ± 0.20	8.87 ± 0.25	N/A	NS	NS
N(mg)	Whole Plant	57.8 ± 3.6	64.9 ± 3.0	66.2 ± 7.4	57.7 ± 1.2	9.2 ± 0.5	NS	11.5
	Stem and Leaves	38.2 ± 3.5	44.2 ± 2.5	47.3 ± 6.4	39.2 ± 1.1	3.0 ± 0.2	NS	10.1
	Root	12.3 ± 1.0	13.3 ± 0.8	11.8 ± 0.9	11.4 ± 0.8	6.1 ± 0.3	NS	2.2
	Nodules	7.3 ± 0.5	7.4 ± 0.3	7.2 ± 0.5	7.1 ± 0.3	N/A	NS	NS

[a] Measures for all plant part(s) expressed as treatment group mean ± SEM.

[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

nodule N concentration (Table 4.8) were found.

4.4.4 *Nitrogen Content*

Whole plant, stem and leaf and root N content (Table 4.8) did not differ among treatments which included *R. leguminosarum* bv. *viceae* inoculation. However, *R. leguminosarum* bv. *viceae* inoculation resulted in greater whole plant and plant part N contents than were found for control plants. No differences among treatments in nodule N content (Table 4.8) were found.

4.4.5 *Root Nodule and N_2 Fixation Measurements*

Root nodule number (Table 4.9) was 24% greater among *R. leguminosarum* bv. *viceae* inoculated plants which also received *B. cereus*. LR+B treated plants had 34% more root nodules than LR-B treated plants while HR+B treated plants had 33% more root nodules than LR-B treated plants.

Individual nodule DM (Table 4.9) was 24% greater for *R. leguminosarum* bv. *viceae* inoculated plants which did not receive *B. cereus*. LR-B treated plants had 29% greater individual nodule dry matter than LR+B and HR+B treated plants. Specific root nodulation, specific nitrogenase activity and nitrogen fixation efficiency (Table 4.9) did not differ among treatments.

Table 4.9: Nodule number, individual nodule DM, specific root nodulation, specific nitrogenase activity and nitrogen fixation efficiency of 34 day old field pea uninoculated, or inoculated with high (HR) or low (LR) rates of *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85 and grown in a 17°C day/12°C night temperature regime.

Parameter[a]	HR+B	HR-B	LR+B	LR-B	C	Sig. Contrasts[b]	LSD Value
Nodule Number $Plant^{-1}$	244 ± 14	212 ± 24	246 ± 20	183 ± 8	0	Bac**	44
Individual Nodule DM (mg)	0.34 ± 0.01	0.40 ± 0.03	0.34 ± 0.03	0.44 ± 0.03	N/A	Bac**	0.08
Specific Root Nodulation[c]	561 ± 55	500 ± 68	599 ± 72	495 ± 54	N/A	NS	NS
Specific Nitrogenase Activity[d]	418.8 ± 61.2	477.7 ± 37.3	535.5 ± 35.9	478.4 ± 38.1	N/A	NS	NS
Nitrogen Fixation Efficiency[e]	0.71 ± 0.02	0.80 ± 0.04	0.81 ± 0.05	0.73 ± 0.04	N/A	NS	NS

[a] Measures for all parameters expressed as treatment group mean ● SEM.

[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

[c] Specific Root Nodulation = nodule number g^{-1} root DM.

[d] Specific Nitrogenase Activity = μmol H_2 evolved in $Ar:O_2$ g^{-1} nodule DM $hour^{-1}$.

[e] N-fix Efficiency = total plant N (mg) $nodule^{-1}$ DM (mg).

4.5 Field Studies - Soybean at the Winnipeg Experimental Site

4.5.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), plant stand density (Table 4.10) was 11% greater for treatments which included inoculation with *B. japonicum* (+R+B and +R-B) versus those which did not include such inoculation (-R+B and -R-B). As well, the plant stand density for the +R+B treatment (*B. japonicum* and *B. cereus* inoculation) was 16%, 17% and 25% greater than that found for the -R-B treatment (no *B. japonicum* or *B. cereus* inoculation), the +R-B treatment (*B. japonicum* inoculation only) and the -R+B treatment (*B. cereus* inoculation only), respectively.

4.5.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

Stem and leaf dry matter yield (DM) (Table 4.10) was 10% greater for treatments that included inoculation with *B. japonicum* versus treatments without such inoculation. +R+B treatment DM was 10% greater than that found for the -R+B treatment and 11% greater than that found for the -R-B treatment. As well, a 49% increase in stem and leaf N concentration (Table 4.10) and 63% increase in stem and leaf N content (Table 4.10) were found for treatments which included *B. japonicum* inoculation.

4.5.3 *Acetylene Reduction Assay at 60 DAP*

The μmols of C_2H_4 produced plant^{-1} hour^{-1} (Table 4.10) was 2830% greater for plants inoculated with *B. japonicum* versus those without such inoculation. No treatment

Table 4.10: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1}) of soybean 60 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	138 ± 1	118 ± 4	111 ± 4	120 ± 4	Rhiz**[c]	9
Stem and Leaf DM (g)	930 ± 31	908 ± 18	843 ± 32	836 ± 16	Rhiz**	75
Stem and Leaf %N	2.38 ± 0.06	2.28 ± 0.11	1.64 ± 0.04	1.50 ± 0.02	Rhiz**	0.24
Stem and Leaf N(g)	22.1 ± 0.9	20.7 ± 1.3	13.8 ± 0.8	12.5 ± 0.3	Rhiz**	3
Nitrogenase Activity	11.84 ± 1.44	10.81 ± 0.81	0.34 ± 0.17	0.43 ± 0.12	Rhiz**	2.78

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

differences due to *B. cereus* inoculation were found. No root nodules were found on plants that had not been inoculated with *B. japonicum*.

4.5.4 *Dry Matter Yield at 90 DAP*

Stem and leaf DM (Table 4.11) was 14% greater for treatments that included *B. cereus* inoculation (+R+B and -R+B) versus those which did not include such inoculation (+R-B and -R-B). Specifically, a 15% increase in stem and leaf DM was found for the +R+B treatment versus the +R-B treatment and a 21% increase was found for the -R+B treatment versus the -R-B treatment. Seed and pod DM (Table 4.11) was 40% greater for treatments that included *B. japonicum* inoculation.

4.5.5 *Nitrogen Concentration at 90 DAP*

Stem and leaf N concentration (Table 4.11) was 54% greater for treatments which included inoculation with *B. japonicum* and 12% greater for treatments which included inoculation with *B. cereus*. Specifically, the +R+B treatment had 13% greater stem and leaf N concentration than the +R-B treatment.

Seed and pod N concentration (Table 4.11) was 37% greater for treatments that included inoculation with *B. japonicum* and 8% greater for treatments that included *B. cereus* inoculation. The +R+B treatment resulted in 6% greater seed and pod N concentration than the +R-B treatment while the -R+B treatment resulted in 10% greater seed and pod N concentration than the -R-B treatment.

Table 4.11: DM (g 3 m^{-1} row), %N and N content (g 3 m^{-1} row) of soybean 90 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	740 ± 33	643 ± 15	687 ± 34	613 ± 43	Bac**[c]	82
	Seed and Pods	693 ± 21	661 ± 40	514 ± 18	452 ± 19	Rhiz**	78
%N	Stem and Leaves	1.40 ± 0.04	1.24 ± 0.04	0.90 ± 0.01	0.81 ● 0.01	Rhiz** Bac**	0.1
	Seed and Pods	3.97 ± 0.10	3.75 ± 0.04	2.95 ± 0.05	2.68 ± 0.04	Rhiz** Bac**	0.21
N(g)	Whole Shoot	37.9 ± 1.8	32.8 ± 1.6	21.4 ± 0.8	17.2 ± 1.1	Rhiz** Bac**	4.1
	Stem and Leaves	10.3 ± 0.5	7.9 ● 0.3	6.2 ● 0.3	5.0 ● 0.4	Rhiz** Bac**	1.2
	Seed and Pods	27.6 ± 1.5	24.9 ± 1.7	15.2 ± 0.5	12.2 ± 0.7	Rhiz** Bac*[d]	3.8

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.

[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

[c] Indicates contrast statements that were significant at P=0.01.

[d] Indicates contrast statements that were significant at P=0.05.

4.5.6 *Nitrogen Content at 90 DAP*

Whole shoot N content (Table 4.11) was 84% greater for treatments which included inoculation with *B. japonicum* and 19% greater for treatments which included inoculation with *B. cereus*. The +R+B treatment had 16% greater whole shoot N content than the +R-B treatment. As well, the -R+B treatment had 24% greater whole shoot N content than the -R-B treatment.

Stem and leaf N content (Table 4.11) was 63% greater for treatments that included *B. japonicum* inoculation and 28% greater for treatments that included *B. cereus* inoculation. The +R+B treatment had 30% greater stem and leaf N content than the +R-B treatment while the -R+B treatment had 25% greater stem and leaf N content than the -R-B treatment.

A 92% increase in seed and pod N content (Table 4.11) was found for treatments which included *B. japonicum* inoculation. As well, a 16% increase in seed and pod N content was found for treatments which included *B. cereus* inoculation.

4.5.7 *Plant and Pod Numbers at 90 DAP*

No differences among treatments for plants $3m^{-1}$ sample of row (Table 4.12) were found. Pods $plant^{-1}$ (Table 4.12) was 18% greater for plants inoculated with *B. japonicum* versus those without such inoculation

Table 4.12: Plants $3m^{-1}$ row and pods $plant^{-1}$ of soybean 90 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plants 3 m^{-1} sample of row	137 ± 3	129 ± 6	132 ± 4	130 ± 4	NS	NS
Pods $plant^{-1}$	13.3 ± 0.1	13.5 ± 0.8	11.6 ± 0.6	11.1 ± 0.6	Rhiz*[d]	2.1

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

4.5.8 *Dry Matter Yield at 120 DAP*

No differences among treatments in stem and leaf dry matter yield (DM) (Table 4.13) were found. Treatments which included inoculation with *B. japonicum* demonstrated a 13% increase in pod wall DM (Table 4.13). The +R-B treatment resulted in a 19% increase in pod DM compared to that found for the -R-B treatment while the +R+B treatment resulted in an 18% increase in pod DM compared to that found for the -R-B treatment.

Seed DM (Table 4.13) was 47% greater for treatments that included *B. japonicum* inoculation. As well, treatments that included *B. cereus* inoculation had 9% greater seed DM. The -R+B treatment demonstrated 23% greater seed DM than the -R-B treatment.

4.5.9 *Nitrogen Concentration at 120 DAP*

No differences among treatments in stem and leaf N concentration (Table 4.13) were found. Pod N concentration (Table 4.13) was 10% greater for treatments that included *B. japonicum* inoculation.

Seed N concentration (Table 4.13) was 27% greater for treatments that included *B. japonicum* inoculation and 6% greater for treatments that included *B. cereus* inoculation. The -R+B treatment resulted in 12% greater seed N concentration compared to the -R-B treatment.

Table 4.13: DM (g 2.5 m^{-1} row), %N and N content (g 2.5 m^{-1} row) of soybean 120 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	222 ± 9	214 ± 7	213 ● 9	205 ± 4	NS	NS
	Pod Walls	214 ± 8	215 ± 8	198 ± 10	182 ± 2	Rhiz**[c]	21
	Seed	458 ± 21	454 ± 19	342 ± 16	278 ● 1	Rhiz** Bac*[d]	37
%N	Stem and Leaves	0.56 ± 0.01	0.53 ± 0.01	0.52 ± 0.02	0.59 ± 0.03	NS	NS
	Pods	0.57 ± 0.02	0.57 ± 0.02	0.52 ± 0.01	0.52 ± 0.01	Rhiz**	0.04
	Seed	6.12 ± 0.08	5.99 ± 0.07	5.03 ± 0.07	4.50 ± 0.04	Rhiz** Bac**	0.15
N(g)	Whole Shoot	30.5 ± 1.7	29.6 ± 1.4	19.4 ± 1.1	14.6 ± 0.2	Rhiz** Bac**	2.7
	Stem and Leaves	1.23 ± 0.07	1.13 ± 0.03	1.13 ± 0.07	1.20 ± 0.07	NS	NS
	Pods	1.23 ± 0.04	1.23 ± 0.06	1.03 ± 0.07	0.90 ± 0.01	Rhiz**	0.11
	Seed	28.1 ± 1.6	27.2 ± 1.4	17.2 ± 1.0	12.5 ± 0.1	Rhiz** Bac**	2.5

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

4.5.10 *Nitrogen Content at 120 DAP*

The N content of whole shoots (Table 4.13) was 77% greater for treatments that included *B. japonicum* inoculation and 13% greater for treatments that included *B. cereus* inoculation. The -R+B treatment was found to have 33% greater whole plant N content that the -R-B treatment. No differences among treatments in stem and leaf N content (Table 4.13) were found. Pod N content (Table 4.13) was 27% greater for treatments that included inoculation with *B. japonicum*. Specifically, the -R+B treatment resulted in a 14% increase in pod N content as compared to the -R-B treatment.

Seed N content (Table 4.13) was 86% greater for treatments that included *B. japonicum* inoculation and 14% greater for treatments that included *B. cereus* inoculation. The -R+B treatment demonstrated 38% greater seed N content that the -R-B treatment.

4.5.11 *Seed Measurements at 120 DAP*

Seed size (250 seed weight) (Table 4.14) was 21% greater for treatments that included *B. japonicum* inoculation and 5% greater for treatments that included *B. cereus* inoculation. The +R+B treatment had 3% greater 250 seed weight than the +R-B treatment while the -R+B treatment had 8% greater 250 seed weight than the -R-B treatment.

Seeds $2.5m^{-1}$ sample of row (Table 4.14) was 22% greater for treatments that included *B. japonicum* inoculation. The -R+B treatment resulted in 14% greater seeds 2.5 m^{-1} sample of row than the -R-B treatment. Seeds $plant^{-1}$ (Table 4.14) was 28%

greater for treatments that included *B. japonicum* inoculation. The +R-B treated plants demonstrated 16%, 27% and 49% increases in seeds plant^{-1} compared to +R+B treated, -R+B treated and -R-B treated plants, respectively. +R+B treated plants demonstrated a 28% increase in seeds plant^{-1} compared to -R-B treated plants.

The Harvest Index (Table 4.14) calculated for treatments that included *B. japonicum* inoculation was 18% greater than that calculated for treatments without such inoculation. As well, Harvest Index was 3% greater for treatments that included inoculation with *B. cereus*. A 9% increase in Harvest Index was found for the -R+B treatment compared to the -R-B treatment.

Table 4.14: 250 seed weight (g), seeds 2.5 m^{-1} row, seeds $plant^{-1}$, and harvest index of soybean 120 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
250 seed weight (g)	38.3 ± 0.5	37.1 ± 0.2	32.4 ± 0.5	30.0 ± 0.3	Rhiz**[c] Bac**	1.1
Seeds $2.5m^{-1}$ sample o f row	3103 ± 128	3178 ± 128	2739 ± 127	2401 ± 19	Rhiz**	302
seeds $plant^{-1}$	28 ± 1	33 ± 1	26 ± 1	22 ± 1	Rhiz**	4
Harvest Index[e]	0.51 ± 0.005	0.51 ± 0.003	0.45 ± 0.006	0.42 ± 0.004	Rhiz** Bac**	0

[a] Measures for all parameter(s) expressed as treatment group mean ● SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.
[e] Harvest index = seed DM (g)/whole plant DM (g).

4.6 Field Studies - Soybean at the Carman Experimental Site

4.6.1 *Plant Stand Density 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.15).

4.6.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

No differences among treatments were found for stem and leaf dry matter yield (DM), stem and leaf N concentration and stem and leaf N content (Table 4.15).

4.6.3 *Acetylene Reduction Assay at 60 DAP*

The μmols of C_2H_4 produced plant^{-1} hour^{-1} (Table 4.15) was 3825% greater for treatments which included inoculation with *B. japonicum* (+R+B and +R-B) versus those without such inoculation (-R+B and -R-B). Specifically, the +R-B treatment (*B. japonicum* inoculation only) demonstrated a 2983% increase in the μmols of C_2H_4 produced plant^{-1} hour^{-1} versus the -R+B treatment (*B. cereus* inoculation only) and a 15741% increase versus the -R-B treatment (no *B. japonicum* or *B. cereus* inoculation). The +R+B treatment (*B. japonicum* and *B. cereus* inoculation demonstrated an 8150% increase in the μmols of C_2H_4 produced plant^{-1} hour^{-1} versus the -R-B treatment. No root nodules were found on plants that had not been inoculated with *B. japonicum*.

Table 4.15: Plant stand density (plants $3m^{-1}$ row), stem and leaf DM (g $3m^{-1}$ row), stem and leaf %N, stem and leaf N content (g $3m^{-1}$ row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1}) of soybean 60 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	65 ± 5	62 ± 3	63 ± 4	69 ±3	NS	NS
Stem and Leaf DM (g)	876 ± 46	859 ± 100	912 ± 50	867 ± 47	NS	NS
Stem and Leaf %N	3.03 ± 0.16	2.90 ± 0.10	2.81 ± 0.11	2.70 ± 0.17	NS	NS
Stem and Leaf N(g)	26.5 ± 1.6	24.8 ± 2.6	25.6 ± 1.5	23.2 ± 1.1	NS	NS
Nitrogenase Activity	4.79 ± 2.65	9.19 ± 1.20	0.30 ± 0.19	0.06 ± 0.04	Rhiz**[c]	4.61

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

4.6.4 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 90 DAP*

No differences among treatments were found for the DM, N concentrations or N contents of stems and leaves, seeds and pods or whole shoots (Table 4.16).

4.6.5 *Plant and Pod Numbers at 90 DAP*

No differences among treatments were found for plants $3m^{-1}$ sample of row (Table 18) or for pods $plant^{-1}$ (Table 4.17).

Table 4.16: DM (g 3 m^{-1} row), %N and N content (g 3 m^{-1} row) of soybean 90 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	591 ± 104	609 ± 108	673 ± 85	518 ± 73	NS	NS
	Seed and Pods	710 ± 82	628 ± 101	695 ± 15	681 ± 35	NS	NS
%N	Stem and Leaves	1.25 ± 0.10	4.84 ± 1.57	6.87 ± 0.45	4.26 ± 1.40	NS	NS
	Seed and Pods	4.10 ± 0.22	4.56 ± 0.59	3.65 ± 0.15	3.70 ± 0.11	NS	NS
N(g)	Whole Shoot	37.1 ± 5.7	59.1 ± 14.3	72.5 ± 8.2	51.4 ± 9.9	NS	NS
	Stem and Leaves	7.6 ± 1.9	30.5 ± 14.1	47.1 ± 8.3	22.9 ± 8.3	NS	NS
	Seed and Pods	29.5 ± 4.4	26.3 ± 3.4	25.4 ± 1.3	25.3 ± 1.9	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ● SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

Table 4.17: Plants 3 m^{-1} row and pods $plant^{-1}$ of soybean 90 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plants 3 m^{-1} sample of row	75 ± 3	77 ± 5	70 ± 5	78 ±7	NS	NS
Pods $plant^{-1}$	23 ± 2	23 ± 1	27 ± 1	24 ± 1	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ● SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

4.6.6 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 120 DAP*

No differences among treatments were found for the DM of stems and leaves, pods or seeds (Table 4.18). As well, the N concentration of stems and leaves and pods (Table 4.18) were not found to differ among treatments. However, seed N concentration (Table 4.18) was found to be 6% higher for treatments that included *B. japonicum* inoculation versus those without such inoculation. No differences among treatments were found for the N content of stems and leaves, pods or seeds (Table 4.18).

4.6.7 *Seed Measurements at 120 DAP*

No differences among treatments were found for 250 seed weights, seeds 2.5 m^{-1} sample of row, seeds $plant^{-1}$ or the harvest indexes calculated for each treatment (Table 4.19).

Table 4.18: DM (g 2.5 m^{-1} row), %N and N content (g 2.5 m^{-1} row) of soybean 120 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	251 ± 18	276 ± 49	281 ± 32	269 ± 21	NS	NS
	Pods	218 ± 8	219 ± 13	226 ± 3	222 ± 5	NS	NS
	Seed	447 ± 21	440 ± 36	439 ± 21	417 ± 20	NS	NS
%N	Stem and Leaves	0.62 ± 0.02	0.58 ± 0.01	0.56 ± 0.03	0.57 ± 0.01	NS	NS
	Pods	0.61 ± 0.03	0.58 ± 0.03	0.61 ± 0.02	0.61 ± 0.02	NS	NS
	Seed	5.99 ± 0.18	5.72 ± 0.10	5.65 ± 0.17	5.41 ± 0.17	Rhiz*[d]	NS
N(g)	Whole Shoot	29.7 ± 2.0	28.0 ± 2.4	27.9 ± 1.9	25.6 ± 1.9	NS	NS
	Stem and Leaves	1.6 ± 0.1	1.6 ± 0.3	1.6 ± 0.2	1.5 ± 0.1	NS	NS
	Pods	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	NS	NS
	Seed	26.8 ± 1.9	25.1 ± 2.0	24.9 ± 1.7	22.7 ± 1.8	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

Table 4.19: 250 seed weight (g), seeds 2.5 m^{-1} row, seeds $plant^{-1}$, and harvest index of soybean 120 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *B. japonicum* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
250 seed weight (g)	36.2 ± 1.5	35.8 ± 1.7	35.2 ± 0.9	33.3 ± 1.0	NS	NS
Seeds 2.5m^{-1} sample of row	3224 ± 108	3189 ± 135	3252 ± 103	3262 ± 93	NS	NS
seeds $plant^{-1}$	67 ± 2	63 ± 5	64 ± 3	54 ± 3	NS	NS
Harvest Index[c]	0.49 ± 0.01	0.47 ± 0.02	0.47 ± 0.02	0.46 ± 0.01	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *B. japonicum* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05
[e] Harvest index = seed DM (g)/whole plant DM (g).

4.7 Field Studies - Common Bean at the Winnipeg Experimental Site

4.7.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.20).

4.7.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

Stem and leaf dry matter yield (DM) (Table 4.20) was 6% and 13% greater for the +R-B treatment (*R. etli* inoculation only) compared to the +R+B treatment (*R. etli* and *B. cereus* inoculation) and the -R-B treatment (no *R. etli* and *B. cereus* inoculation), respectively. The -R+B treatment (*B. cereus* inoculation only) demonstrated an 11% increase in stem and leaf DM yield versus the -R-B treatment.

Stem and leaf N concentration (Table 4.20) did not differ among treatments. The +R-B treatment resulted in 11% and 13% greater stem and leaf N content (Table 4.20) compared to the +R+B and -R-B treatments, respectively.

4.7.3 *Acetylene Reduction Assay at 60 DAP*

No differences among treatments were found for the μmols of C_2H_4 produced plant^{-1} hour^{-1} (Table 4.20).

Table 4.20: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1} of common bean 60 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	34 ± 1	31 ± 1	32 ± 2	36 ± 2	NS	NS
Stem and Leaf DM (g)	617 ± 18	656 ± 10	647 ± 16	581 ± 8	NS	36
Stem and Leaf %N	2.42 ± 0.11	2.51 ± 0.17	2.43 ± 0.15	2.51 ± 0.16	NS	0.16
Stem and Leaf N(g)	14.9 ± 1.0	16.4 ± 0.9	15.7 ± 0.8	14.6 ± 1.1	NS	1.2
Nitrogenase Activity	1.58 ± 0.29	5.53 ± 2.22	2.57 ± 0.56	2.15 ± 0.70	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ● SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

4.7.4 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 90 DAP*

Stem and leaf DM (Table 4.21) was 10% greater for treatments which included inoculation with *R. etli* versus those without such inoculation. No differences among treatments were found for seed and pod DM (Table 4.21).

No differences among treatments were found for the N concentration of stems and leaves or seed and pods (Table 4.21). As well, no differences among treatments were found for the N content of whole shoots, stems and leaves or seeds and pods (Table 4.21).

4.7.5 *Plant and Pod Numbers at 90 DAP*

No differences among treatments were found for plants 3 m^{-1} sample of row or for pods $plant^{-1}$ (Table 4.22).

Table 4.21: DM (g 3 m^{-1} row), %N and N content (g 3 m^{-1} row) of common bean 90 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	375 ± 19	369 ± 19	344 ± 16	333 ± 6	Rhiz[c,d]	NS
	Seed and Pods	604 ± 16	588 ± 33	596 ± 28	566 ± 33	NS	NS
%N	Stem and Leaves	1.96 ± 0.13	1.97 ± 0.13	2.00 ± 0.10	1.96 ± 0.13	NS	NS
	Seed and Pods	2.44 ± 0.13	2.57 ± 0.14	2.58 ± 0.12	2.55 ± 0.13	NS	NS
N(g)	Whole Shoot	22.0 ± 1.2	22.6 ± 2.4	22.4 ± 1.8	21.1 ± 2.0	NS	NS
	Stem and Leaves	7.3 ± 0.3	7.3 ± 0.8	6.9 ± 0.6	6.6 ± 0.5	NS	NS
	Seed and Pods	14.7 ± 0.9	15.3 ± 1.7	15.5 ± 1.4	14.5 ± 1.5	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

Table 4.22: Plants 3 m^{-1} row and pods $plant^{-1}$ of common bean 90 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plants 3 m^{-1} sample of row	39 ± 1	37 ± 1	41 ± 2	37 ± 1	NS	NS
Pods $plant^{-1}$	17.5 ± 0.5	18.7 ± 0.6	16.8 ± 0.8	17.7 ± 0.6	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

4.7.6 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 120 DAP*

Stem and leaf DM yield (Table 4.23) was 11% greater for treatments that included inoculation with *R. etli* as compared to treatments without such inoculation. No differences among treatments were found for pod or seed DM yield (Table 4.23).

No differences among treatments were found for the N concentration of stems and leaves, pods or seeds (Table 4.23). As well, no differences among treatments were found for the N content of whole plants, stem and leaves, pod or seeds (Table 4.23).

4.7.7 *Seed Measurements at 120 DAP*

No differences among treatments were found for 250 seed weight, seeds 2.5 m^{-1} sample of row, seeds $plant^{-1}$ or for the harvest indexes calculated for each treatment (Table 4.24).

Table 4.23: DM (g 2.5 m^{-1} row), %N and N content (g 2.5 m^{-1} row) of common bean 120 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	185 ± 7	194 ± 2	170 ± 4	172 ± 8	Rhiz**[c]	NS
	Pods	166 ± 5	172 ± 5	158 ± 4	159 ± 8	NS	NS
	Seed	432 ± 5	446 ± 12	407 ± 19	414 ± 30	NS	NS
%N	Stem and Leaves	0.66 ± 0.02	0.67 ± 0.01	0.70 ± 0.02	0.68 ± 0.02	NS	NS
	Pods	1.80 ± 0.05	1.76 ± 0.05	1.80 ± 0.06	1.76 ± 0.08	NS	NS
	Seed	3.47 ± 0.06	3.51 ± 0.10	3.48 ± 0.12	3.46 ± 0.16	NS	NS
N(g)	Whole Shoot	18.8 ± 0.8	20.0 ± 0.9	18.3 ± 1.3	18.4 ± 1.9	NS	NS
	Stem and Leaves	1.09 ± 0.18	1.30 ± 0.02	1.19 ± 0.04	1.17 ± 0.07	NS	NS
	Pods	2.6 ± 0.4	3.0 ± 0.1	2.8 ± 0.1	2.8 ± 0.2	NS	NS
	Seed	15.0 ± 0.4	15.7 ± 0.8	14.3 ± 1.1	14.4 ± 1.6	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.

[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.

[c] Indicates contrast statements that were significant at P=0.01.

[d] Indicates contrast statements that were significant at P=0.05

Table 4.24: 250 seed weight (g), seeds 2.5 m^{-1} row, seeds $plant^{-1}$, and harvest index of common bean 120 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
250 seed weight (g)	40.0 ± 0.7	39.6 ± 1.0	38.8 ± 1.0	39.2 ± 1.3	NS	NS
Seeds $2.5m^{-1}$ sample	2809 ± 61	2921 ± 45	2723 ± 80	2733 ± 129	NS	NS
seeds $plant^{-1}$	89 ± 5	92 ± 6	85 ± 4	71 ± 7	NS	NS
Harvest Index[c]	0.55 ± 0.01	0.55 ± 0.01	0.55 ± 0.01	0.56 ± 0.01	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05
[e] Harvest index = seed DM (g)/whole plant DM (g).

4.8 Field Studies - Common Bean at the Carman Experimental Site

4.8.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.25).

4.8.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

No differences among treatments were found for stem and leaf dry matter (DM) yield, stem and leaf N concentration or stem and leaf N content (Table 4.25).

4.8.3 *Acetylene Reduction Assay at 60 DAP*

No differences among treatments were found for the μmols of C_2H_4 produced plant^{-1} hour^{-1} (Table 4.25).

Table 4.25: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1} of common bean 60 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	21 ± 1	20 ± 1	21 ± 1	18 ± 2	NS	NS
Stem and Leaf DM (g)	562 ± 50	569 ± 32	581 ± 31	548 ± 29	NS	NS
Stem and Leaf %N	3.62 ± 0.12	3.51 ± 0.10	3.43 ± 0.10	3.58 ± 0.08	NS	NS
Stem and Leaf N(g)	20.1 ± 1.3	20.1 ± 1.4	20.0 ± 1.6	19.5 ± 0.6	NS	NS
μmols C_2H_4 produced $plant^{-1}$ $hour^{-1}$	1.97 ± 0.32	1.12 ± 0.68	1.26 ± 0.86	1.59 ± 1.11	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

4.8.4 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 90 DAP*

No differences among treatments were found for the DM yield (Table 4.26), N concentration (Table 4.26) or N content (Table 4.26) of whole shoots, stems and leaves, seeds and pods.

4.8.5 *Plant and Pod Numbers at 90 DAP*

No differences among treatments for plants 3 m^{-1} sample of row (Table 4.27) or for pods $plant^{-1}$ (Table 4.27) were found.

Table 4.26: DM (g 3 m^{-1} row), %N and N content (g 3 m^{-1} row) of common bean 90 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	420 ● 39	371 ± 48	390 ± 27	462 ± 54	NS	NS
	Seed and Pods	663 ± 45	651 ± 18	722 ± 35	756 ± 60	NS	NS
%N	Stem and Leaves	2.08 ± 0.16	1.86 ± 0.09	1.94 ± 0.06	2.05 ± 0.06	NS	NS
	Seed and Pods	3.15 ± 0.10	3.14 ± 0.14	2.99 ± 0.03	3.12 ± 0.09	NS	NS
N(g)	Whole Shoot	29.7 ± 1.3	27.3 ± 1.6	29.2 ± 1.7	33.0 ± 2.7	NS	NS
	Stem and Leaves	8.9 ± 1.4	6.9 ± 1.1	7.6 ± 0.7	9.6 ± 1.3	NS	NS
	Seed and Pods	20.8 ± 1.2	20.4 ± 0.8	21.6 ● 1.2	23.5 ± 1.4	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ● SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

Table 4.27: Plants 3 m^{-1} row and pods $plant^{-1}$ of common bean 90 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plants 3 m^{-1} sample of row	20 ± 2	19 ± 1	21 ± 1	18 ± 2	NS	NS
Pods $plant^{-1}$	37.5 ± 5.4	35.5 ● 2.6	32.3 ± 3.0	42.4 ± 1.8	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

4.8.6 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 120 DAP*

No differences among treatments were found for the DM yields of stems and leaves, pods and seeds (Table 4.28). As well, the N concentration of stems and leaves and seed (Table 4.28) did not differ among treatments. However, pod N concentration (Table 4.28) was found to be 10% higher for treatments that did not include inoculation with *R.. etli* as compared to those that did include such inoculation.

No differences among treatments were found for the N content of whole plants, stems and leaves, pods or seeds.

4.8.7 *Seed Measurements at 120 DAP*

No differences among treatments were found for 250 seed weight, seed 2.5 m^{-1} sample of row or seeds $plant^{-1}$ (Table 4.29). Harvest index was 3% greater for treatments that did not receive inoculation with *R. etli* versus treatments that did receive such inoculation. As well, harvest index was 1% greater for treatments that did not receive inoculation with *B. cereus* as compared to treatments that did receive such inoculation. Specifically, harvest index was 5% greater for the +R-B, -R+B and -R-B treatments compared to the +R+B treatment.

Table 4.28: DM (g 2.5 m^{-1} row), %N and N content (g 2.5 m^{-1} row) of common bean 120 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter	Plant Part(s)[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
DM (g)	Stem and Leaves	248 ± 18	209 ± 7	207 ± 29	218 ± 14	NS	NS
	Pods	199 ± 14	189 ± 10	190 ± 17	196 ± 5	NS	NS
	Seed	502 ± 31	491 ± 21	495 ± 47	514 ± 7	NS	NS
%N	Stem and Leaves	0.83 ± 0.06	0.75 ± 0.01	0.81 ± 0.09	0.81 ± 0.05	NS	NS
	Pods	1.37 ± 0.09	1.39 ± 0.09	1.50 ± 0.09	1.52 ± 0.10	Rhiz*[d]	NS
	Seed	4.22 ± 0.11	4.26 ± 0.12	4.16 ± 0.12	4.23 ± 0.09	NS	NS
N(g)	Whole Shoot	26.0 ± 1.5	26.2 ± 1.5	25.0 ± 2.4	26.9 ± 0.2	NS	NS
	Stem and Leaves	2.1 ± 0.3	1.6 ± 0.1	1.7 ± 0.4	1.8 ± 0.2	NS	NS
	Pods	2.8 ± 0.4	2.7 ± 0.3	2.9 ± 0.3	3.0 ± 0.2	NS	NS
	Seed	21.1 ± 1.0	20.8 ± 0.6	20.3 ± 2.1	21.8 ± 0.4	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05

Table 4.29: 250 seed weight (g), seeds 2.5 m^{-1} row, seeds $plant^{-1}$, and harvest index of common bean 120 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. etli* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
250 seed weight (g)	44.1 ± 0.5	44.8 ± 0.5	43.6 ± 0.7	45.0 ± 0.8	NS	NS
Seeds 2.5 m^{-1} sample	2981 ± 163	2872 ± 139	2969 ± 261	3001 ± 62	NS	NS
seeds $plant^{-1}$	187 ± 15	138 ● 14	180 ± 6	222 ± 39	NS	NS
Harvest Index[e]	0.53 ± 0.008	0.56 ± 0.005	0.56 ± 0.005	0.56 ± 0.007	Rhiz*[d] Bac*[d]	0

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. etli* inoculation.
Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05
[e] Harvest index = seed dry matter yield (g)/whole plant dry matter yield (g).

4.9 Field Studies - Field Pea at the Winnipeg Experimental Site

4.9.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.30).

4.9.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

No differences among treatments were found for stem and leaf dry matter yield (DM), stem and leaf N concentration and stem and leaf N content (Table 4.30).

4.9.3 *Acetylene Reduction Assay at 60 DAP*

No differences among treatments were found for the μmols of C_2H_4 produced $plant^{-1}$ $hour^{-1}$ (Table 4.30).

Table 4.30: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1} of field pea 60 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	62 ± 1	62 ± 2	66 ± 1	66 ± 2	NS	NS
Stem and Leaf DM (g)	374 ± 6	383 ± 10	394 ± 9	397 ± 11	NS	NS
Stem and Leaf %N	3.83 ± 0.09	3.83 ± 0.05	3.83 ± 0.05	3.82 ± 0.04	NS	NS
Stem and Leaf N(g)	14.3 ± 0.4	14.7 ± 0.6	15.1 ± 0.20	15.2 ± 0.5	NS	NS
Nitrogenase Activity	1.19 ± 0.70	1.44 ± 0.59	1.00 ± 0.32	1.76 ± 0.99	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

4.10 Field Studies - Field Pea at the Carman Experimental Site

4.10.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.31). Due to an equipment malfunction no data for +R-B treated field pea could be collected.

4.10.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

No differences among treatments were found for stem and leaf dry matter yield (DM), stem and leaf N concentration and stem and leaf N content (Table 4.31). Due to an equipment malfunction, no data for +R-B treated field pea could be collected.

4.10.3 *Acetylene Reduction Assay at 60 DAP*

No differences among treatments were found for the μmols of C_2H_4 produced $plant^{-1}$ $hour^{-1}$ (Table 4.31). Due to an equipment malfunction no data for +R-B treated field pea could be collected.

Table 4.31: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1} of field pea 60 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	-R+B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	53 ± 2	N/A	52 ± 1	54 ● 6	N/A	NS
Stem and Leaf DM (g)	470 ± 19	N/A	501 ± 15	465 ± 27	N/A	NS
Stem and Leaf %N	4.17 ± 0.12	N/A	4.20 ± 0.06	4.04 ± 0.07	N/A	NS
Stem and Leaf N(g)	19.7 ± 1.3	N/A	21.1 ± 0.8	18.8 ± 1.0	N/A	NS
Nitrogenase Activity	4.13 ± 1.57	N/A	7.75 ± 1.15	6.49 ± 2.32	N/A	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

4.11 Field Studies - Lentil at the Winnipeg Experimental Site

4.11.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.32).

4.11.2 *Dry Matter Yield, Nitrogen Concentration and Nitrogen Content at 60 DAP*

No differences among treatments were found for stem and leaf dry matter yield (DM), stem and leaf N concentration and stem and leaf N content (Table 4.32).

4.11.3 *Acetylene Reduction Assay at 60 DAP*

No differences among treatments were found for the μmols of C_2H_4 produced plant^{-1} hour^{-1} (Table 4.32).

Table 4.32: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1} of lentil 60 DAP at the Winnipeg site in 1994 which were inoculated with (+R) or without (-R) *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	75 ± 5	85 ± 3	75 ± 4	75 ± 4	NS	NS
Stem and Leaf DM (g)	526 ± 21	534 ± 13	511 ± 23	487 ± 15	NS	NS
Stem and Leaf %N	2.76 ± 0.09	2.84 ± 0.06	2.87 ± 0.04	2.93 ± 0.05	NS	NS
Stem and Leaf N(g)	14.6 ± 1.0	15.1 ± 0.5	14.7 ± 0.8	14.3 ± 0.6	NS	NS
Nitrogenase Activity	1.46 ± 0.43	2.33 ± 0.49	2.46 ± 0.90	2.61 ± 1.52	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

4.12 Field Studies - Lentil at the Carman Experimental Site

4.12.1 *Plant Stand Density at 60 DAP*

At 60 days after planting (DAP), no differences among treatments were found for plant stand density (Table 4.33).

4.12.2 *Dry Matter Yield, Nitrogen Concentrations and Nitrogen Content at 60 DAP*

No differences among treatments were found for stem and leaf dry matter yield (DM), stem and leaf N concentration and stem and leaf N content (Table 4.33).

4.12.3 *Acetylene Reduction Assay at 60 DAP*

No differences among treatments were found for the μmols of C_2H_4 produced $plant^{-1}$ $hour^{-1}$ (Table 4.33).

Table 4.33: Plant stand density (plants 3 m^{-1} row), stem and leaf DM (g 3 m^{-1} row), stem and leaf %N, stem and leaf N content (g 3 m^{-1} row) and nitrogenase activity (μmols C_2H_4 $plant^{-1}$ h^{-1} of lentil 60 DAP at the Carman site in 1994 which were inoculated with (+R) or without (-R) *R. leguminosarum* bv. *viceae* and with (+B) or without (-B) *B. cereus* UW85.

Parameter[a]	+R+B	+R-B	-R+B	-R-B	Sig. Contrasts[b]	LSD Value
Plant Stand Density	80 ± 7	82 ± 4	73 ± 7	81 ± 6	NS	NS
Stem and Leaf DM (g)	394 ± 29	352 ± 10	377 ± 11	408 ± 22	NS	NS
Stem and Leaf %N	3.25 ± 0.05	3.18 ± 0.04	3.22 ± 0.06	3.23 ± 0.05	NS	NS
Stem and Leaf N(g)	12.8 ± 0.8	11.2 ± 0.4	12.1 ± 0.2	13.2 ± 0.6	NS	NS
Nitrogenase Activity	3.55 ± 1.56	2.60 ± 1.26	2.41 ± 0.70	2.75 ± 1.22	NS	NS

[a] Measures for all parameter(s) expressed as treatment group mean ± SEM.
[b] Rhiz indicates contrast statements testing effects of *R. leguminosarum* inoculation. Bac indicates contrast statements testing effects of *B. cereus* (UW85) inoculation.
[c] Indicates contrast statements that were significant at P=0.01.
[d] Indicates contrast statements that were significant at P=0.05.

5.0 Discussion

In this thesis, it was hypothesized that the *Bacillus cereus* strain UW85 (here on referred to as UW85) would promote the growth and yield of plants in the growth chamber and in the field. Furthermore, it was hypothesized that use of this strain would result in increased plant tissue N content, nitrogenase activity and root nodule numbers in treated plants. Central to these hypotheses was the concept that UW85 would have a positive effect on growth.

Four major areas of plant growth promotion will be considered in this discussion. These include: 1) the promotion of plant stand density 2) the promotion of plant growth and seed yield 3) the promotion of plant tissue N concentration and N content and 4) the promotion of nitrogenase activity and root nodule numbers. As well, the possible mechanisms by which UW85 functions to produce growth promotions and the commercial potential this organism may have in current agricultural production systems will be discussed.

5.1 Promotion of Plant Stand Density

For soybean, 100% of seeds planted emerged, regardless of treatment, at 10 days after planting (DAP) in a controlled-environment study. In the two field pea studies conducted at different temperature regimes (22°C day/17°C night and 17°C day/12°C night), no appreciable treatment differences in emergence were found with between 92 and 100% of seeds planted successfully emerging at 10 DAP. In the common bean study, 75 to 100% of seeds not treated with UW85 emerged while only between 54 and 58% of seeds treated with the strain emerged at 10 DAP. UW85-treated common bean seedlings were noted as having pruned and blackened roots and brown cotyledon spots. Other cases of UW85 causing such damage or inhibiting seedling emergence have not been reported in the literature. In fact, there are no instances in the literature of UW85 being tested with common bean or field pea previous to this work.

The lack of effect of UW85 on soybean seedling emergence under gnobiotic conditions in controlled-environment studies has not been reported in past work. However, laboratory screening trials have demonstrated that UW85 is able to protect alfalfa seedlings from damping-off caused by *Phytophthora megasperma* f. sp. *medicagnis*, *Pythium* sp. and *Aphanomyces* sp. (Handelsman et al. 1988, Handelsman et al. 1990, Silo-Suh et al. 1994), soybean seedlings from damping-off caused by *Phytophthora* sp. (Handelsman et al. 1988) and tobacco seedlings from damping-off caused by *Phytophora parastica* var. *nicotianae* (Handelsman et al. 1991). As well, two fungistatic antibiotics have been purified from UW85 culture and shown to suppress damping-off in alfalfa caused by *Phytophthora medicaginis* (Silo-Suh et al. 1994). Based

on this, it is reasonable to assume that UW85 can play a role in promoting seedling emergence by acting as a bio-control agent in some host/pathogen relationships. Our work demonstrated no promotion of soybean emergence due to UW85 inoculation of seeds in the absence of disease. There are cases in the literature where promotions of seedling emergence were found in the absence of pathogens. For example, Kloepper et al. (1986) identified several strains from the *Pseudomonas* genus which repeatedly increased the emergence of soybean seedlings in cold soil to an average level at least 50 % greater than controls. This happened even when steps were taken to eliminate the effects of any "minor pathogens" present and no visible symptoms of disease were noted.

At the Carman site, no differences in plant stand density at 60 DAP or 90 DAP were found. At the Winnipeg site, the plant stand density at 60 DAP was between 16 to 25% greater for soybean inoculated with both *B. japonicum* and UW85 (+R+B) versus all other treatments. However, plant stand density at 90 DAP did not differ among treatments. Measurements of common bean, field pea and lentil plant stand density at 60 DAP and common bean plant stand density at 90 DAP, revealed no differences among treatments at either field site.

In a 1987 experiment, Halverson and Handelsman (1991) and Halverson (1991) found that the seedling emergence of UW85-treated soybean at 28 DAP was 33 to 40% greater compared to controls. These increases were still apparent at 126 DAP. Similar field experiments conducted in 1986 and 1989 did not demonstrate any improvements in seedling emergence. UW85 has also been documented as increasing the emergence of alfalfa and soybean seedlings by 50% as compared to controls (Halverson et al. 1988)

and, in another case, as increasing the emergence of alfalfa seedlings by 67% as compared to controls (Handelsman 1990). Osburn et al. (1995), attributed enhancements in soybean seedling emergence seen in one year of a five year series of field studies to UW85. At their Whitewater site in 1990 at two weeks after planting, UW85 inoculation resulted in 27 to 108% greater soybean seedling counts while, at four weeks after planting, the increases ranged from 22 to 96%. At their Racine site, at four weeks after planting, an 82% increase in seedling count was found.

In work on plant stand density and UW85 documented in the literature, increases in density were often clearly attributed to UW85. It was noted by Halverson (1991) that his seed coating method of inoculating soybean with UW85 may have led to osmotic priming of the seed but that this did not explain plant stand density changes seen at 28 DAP. In the case of Osburn et al. (1995), formulations of UW85 that increased seedling emergence included both seed-applied peat powder and in-furrow granular formulations.

In my thesis field research, the treatment which included UW85 and *B. japonicum* inoculation (+R+B) resulted in increased soybean plant stand density while the treatment that included only UW85 (-R+B) did not result in increased plant stand density. It would appear that *B. japonicum* played a role in promoting seedling emergence when used with UW85. Past field studies with soybean and UW85 have never documented this. Chanway et al. (1989) documented 33% greater emergence for lentil (cv. Eston) inoculated with *Rhizobium leguminosarum* bv. *viceae* strain 175P1 versus uninoculated lentil. When lentil were co-inoculated with *R. leguminosarum* bv. *viceae* and *Pseudomonas putida* strain G11-32, 23% greater emergence versus lentil inoculated with

only the *R. leguminosarum* bv. *viceae* strain and 64% greater emergence versus uninoculated lentil was found. Unfortunately, a statistical analysis of increases in seedling emergence involving comparisons with uninoculated controls was not provided by Chanway et al. (1989).

Later measures of soybean plant stand density at Winnipeg revealed no differences among treatments. UW85 appeared to have an influence on plant stand density at 60 DAP but this influence did not continue through the growing season. In past research, UW85 has demonstrated inconsistent performance in emergence promotion across sites and years. This thesis demonstrates that inconsistent performance can also be found during the growing season at a given site on a given year. The fact that no plant stand density increases were seen at the Carman site is not surprising considering the past inconsistent performance of UW85 .

Field testing of UW85 with common bean, field pea and lentil has not been documented in the literature. In my research, UW85 showed no effect on the seedling emergence of these crops. UW85 has been shown to be effective in the field in promoting the seedling emergence of soybean and alfalfa as previously noted. Examples exist of PGPR being effective with only certain species of crops or even certain cultivars of given species (Chanway et al. 1989). Based on both the laboratory and field results generated in this thesis, it is felt that the plant stand density of common bean, field pea and lentil is not promoted through the use of UW85. However, repeated testing in a variety of environments could prove otherwise. Significant to note is that although common bean seedlings were damaged by UW85 inoculation in controlled-environment

studies, that same damage did not appear to occur in the field.

5.2 Promotion of Plant Growth and Yield

The promotion of plant growth and seed or grain yield due to PGPR use have been two of the most commonly measured parameters in studies dealing with the effects of PGPR (Glick 1995; Kloepper et al. 1989; Brown 1974). In our work, soybean grown in a controlled-environment study for 34 days demonstrated several such promotions. Total leaf area and plant height were each 19% greater for plants inoculated with higher rates of *B. japonicum* and UW85 (HR+B) versus those inoculated with higher rates of *B. japonicum* alone (HR-B). As well, HR+B treated plants demonstrated whole plant DM, stem and leaf DM, root DM and total nodule DM that were 24%, 27%, 18% and 26% greater, respectively. Halverson and Handelsman (1991) and Halverson (1991) have shown that UW85 inoculation resulted in a 33% increase in the nodule mass of soybean in controlled-environment studies. It should be noted that my thesis is the first time, in the context of controlled-environment studies, that UW85-induced growth promotions in plant parameters other than nodule DM have been reported .

That specific PGPR strains can result in a diversity of responses depending on plant species and cultivar has been documented in the literature (Chanway et al. 1989; Sarig et al.1986; Li and Alexander 1988; Handelsman et al. 1990). However, this thesis is the first time that UW85-mediated promotions in plant growth were investigated in species other than soybean and alfalfa. Field pea tested with UW85 in a temperature regime of 22°C day/17°C night demonstrated that plants inoculated with lower rates of *R. leguminosarum* and UW85 (LR+B) had 22% lower whole plant DM than plants inoculated with lower rates of *R. leguminosarum* alone (LR-B). Specifically,

LR+B treated plants had lower root and nodule DM than LR-B treated plants. Since field pea tested in a temperature regime of 17°C day/12°C night and common bean did not demonstrate any growth promotions due to UW85, my research has demonstrated that UW85 does not have universally positive effects on plant growth across species. There are no previous reports of negative effects of UW85 on plant growth in controlled-environment studies, however Osburn et al. (1995) reported some cases of negative effects on soybean seed yield in the field.

In field experiments involving soybean, promotions of plant growth and yield were found at the Winnipeg site. At 90 DAP, stem and leaf DM was 15% greater for plants inoculated with both *B. japonicum* and UW85 (+R+B) versus plants inoculated with *B. japonicum* alone (+R-B) and 21% greater for plants inoculated with only UW85 (-R+B) versus plants that received no inoculations (-R-B). At 120 DAP, final seed yield was 23% greater for -R+B treated plants versus -R-B treated plants. Seed size had increased for +R+B treated plants compared to +R-B treated plants aw well as for -R+B treated plants compared to -R-B treated plants. The increases in seed yield and seed size for -R+B treated plants versus -R-B treated plants were reflected in a 9% increase in harvest index for -R+B treated plants. No promotions of soybean plant growth or yield due to UW85 inoculation were found at the Carman site. It should be noted that common bean at the Winnipeg site inoculated with UW85 (-R+B) had 10% greater stem and leaf DM than uninoculated common bean (-R-B) at 60 DAP. UW85-mediated promotions of plant growth in the field with species other than soybean or alfalfa have not been demonstrated previously in the literature.

Growth promotions in soybean due to the use of UW85 have been previously demonstrated. Halverson (1991), in a 1987 experiment, found that final mean seed yields were 38 to 50% greater for UW85 treated plants versus untreated plants (P=0.10) while no differences in seed yield among treatments were found in a similar 1989 experiment. Increases in shoot height at 49 and 63 DAP for UW85 treated plants in the 1987 experiment was also noted (P=0.10). Osburn et al. (1995), in a series of experiments at two sites over a five year period, demonstrated several instances of increased seed yield for three different soybean cultivars due to the use of various formulations of UW85 (P=0.10). However, when the data was re-examined at P=0.05 using LSD values provided by the authors, far fewer instances of growth promotion were found. At their Racine site, a single instance of a 139% increase in seed yield due to UW85 use occurred. At their Whitewater site, there were eight instances of seed yield promotion due to the use of UW85. Increases ranged from 14 to 23%.

The limited work done with UW85 in field research has demonstrated that seed yield increases are not consistent across experimental sites and test years. The research done in this thesis confirms that fact. However, it is important to note that seed yield increases did occur. The work in this thesis, unlike previous work, has also demonstrated promotions in stem and leaf DM, seed size and harvest index due to the use of UW85.

5.3 Promotion of Plant Tissue N Concentration and N Content

There are relatively few reports of the effects of PGPR on plant tissue N concentration and N content (Sarig et al.1986; Alagawadi and Gaur 1988; Li and Alexander 1988; Knight and Langston-Unkefer 1988; Azcon 1993). This thesis is the first report of UW85-induced promotions for any species. In the controlled-environment study involving soybean, plants that were inoculated with *B. japonicum*, but without UW85 (HR-B), demonstrated root N concentrations that were greater than all other treatments. No other plant part differences in N concentration were found. However, plants inoculated with higher rates of *B. japonicum* and UW85 (HR+B) demonstrated 22% greater total nodule N content than plants inoculated with *B. japonicum* alone (HR-B). As well, HR+B treated plants were found to have 15% greater whole plant N content than HR-B plants. It was noted previously that soybean that received the HR+B treatment had greater root DM compared to HR-B treated plants. It appears that this DM increase resulted in a "dilution effect" causing a corresponding decrease in root N concentration.

In the controlled-environment study involving field pea in a 22°C day/17°C night temperature regime, plants inoculated with lower rates of *R. leguminosarum* bv. *viceae* and UW85 (LR+B) demonstrated greater whole plant and stem and leaf N concentrations than plants inoculated with lower rates of *R. leguminosarum* bv. *viceae* and without UW85 (LR-B). However, as noted previously, LR+B treated plants were found to have lower whole plant DM than LR-B treated plants. Also, the LR+B treatment resulted in 20% lower root N content than the LR-B treatment which was accompanied by 36%

lower root DM than the LR-B treatment. Hence, the negative effect of the LR+B treatment on DM accumulation was the cause of the increases in plant tissue N concentration observed.

It could be suggested that changes in N content and concentration found were being influenced in large part by promotions in plant DM. Increased root DM for HR+B treated soybean could have diluted the concentration of N in root tissues leading to higher N concentrations, but lower N content, for HR-B treated plants. But, the same scenario was not found between plants inoculated with lower rates of *B. japonicum* and UW85 (LR+B) and plants inoculated with lower rates of *B. japonicum* alone (LR-B). LR+B plants had 22% greater root DM than LR-B treated plants while no differences were found in root N concentration and content. In field pea, lower root N content and root DM occurred for the LR+B treatment compared to the LR-B treatment while no differences in root N concentration were found.

Controlled-environment studies addressing the promotion of N concentration and N content of plant parts by PGPR documented in the literature prove to be as variable as the results presented in my thesis. Sarig et al. (1986) found that vetch inoculated with certain *Azospirillum brailense* strains demonstrated greater shoot and root N concentrations and greater shoot and root DM although no promotions were observed with garden peas. Alagawadi and Gaur (1988) found that promotions in N and P content of chickpea grain and straw attributed to *Pseudomonas striata* or *Bacillus polymyxa* inoculation mimicked promotions in grain yield and straw DM. However, Li and Alexander (1988), while attributing DM promotions in alfalfa to a *Pseudomonas* species

found no increase in the N content of plant parts. Knight and Langston-Unkefer (1988) demonstrated a promotion in whole plant N content of alfalfa inoculated with a different *Pseudomonas* species. In other work with *Pseudomonads*, Azcon (1993) demonstrated increased N content in sulla clover shoots due to PGPR use.

In the field, promotion of the N concentration and N content of plant parts due to UW85 inoculation occurred with soybean at the Winnipeg experimental site. At 90 DAP, soybean inoculated with *B. japonicum* and UW85 (+R+B) had 13% greater stem and leaf N concentration and 6% greater seed and pod N concentration than that found for soybean inoculated with *B. japonicum* alone (+R-B). Seed and pod N concentration was also 10% greater for soybean inoculated with UW85 alone (-R+B) versus soybean that received no inoculations (-R-B). At 120 DAP, seed N concentration was 12% greater in the case of the -R+B treatment compared to the -R-B treatment. Interestingly, these increases in N concentration corresponded to increases, rather than decreases, in plant part DM. Whole shoot N content at 90 DAP was 16% greater for the +R+B treatment versus the +R-B treatment and 24% greater for the -R+B treatment versus the -R-B treatment. Stem and leaf N content at 90 DAP was 30% greater for the +R+B treatment versus the +R-B treatment and 25% greater for the -R+B treatment versus the -R-B treatment. Treatments including UW85 inoculation resulted in a 16% increase in seed and pod N content. At 120 DAP, the -R+B treated soybean demonstrated increases of 33%, 14% and 38% in whole shoot, pod and seed N content, respectively compared to the -R-B treated soybean.

Unfortunately, corresponding laboratory or greenhouse experiments and field

experiments investigating PGPR effects on plant part N concentration and N content are relatively rare in the literature. Sarig et al. (1986) found that N concentrations of vetch shoots were greater due to *A. brasilense* inoculation both in the laboratory and field studies. Raverkar and Konde (1988) found that whole plant N concentration was greater in the field for the peanut cultivar Robut 33-1 when inoculated with an *A. lipoferum* strain alone compared to controls or plants inoculated with the suspected PGPR and a *Rhizobium* sp. Stem and leaf N content was also found to be greater with only PGPR inoculation. My field research with soybean also demonstrated that PGPR inoculation alone resulted in plant tissue N concentration and N content promotions.

5.4 Promotion of Nitrogenase Activity and Root Nodule Number

As with promotions of plant growth and yield, the areas of nitrogenase activity and root nodule number have received significant attention in the scientific literature (Brown 1974; Kloepper et al. 1989; Kapulnik 1991; Beauchamp 1993). In my soybean controlled-environment study, plants inoculated with higher rates of *B. japonicum* and UW85 (HR+B) had 27% more root nodules than plants inoculated with higher rates of *B. japonicum* alone (HR-B). This corresponds with previous work done with UW85 (Halverson and Handelsman 1991; Halverson 1991). Plants inoculated with lower rates of *B. japonicum* did not demonstrate any UW85-mediated differences in root nodule number. However, when specific root nodulation rates were calculated, no differences among treatments due to *B. japonicum* or UW85 inoculation status were found. It is felt that increases in nodule number per plant were due to increased root mass and not to a specific stimulation of the nodulation process. Other controlled-environment studies using UW85 and measuring nodulation have not documented this (Halverson and Handelsman 1991; Halverson 1991). Halverson (1991), in a field study, showed that UW85 inoculation of soybean resulted in plants with greater numbers of nodules on their primary and lateral roots as well as longer roots than plants which did not receive UW85 inoculation.

In past controlled environment work regarding nitrogenase activity and UW85 (Halverson and Handelsman 1991; Halverson 1991) nitrogenase activity per plant was 58 to 71% greater for soybean inoculated with UW85 versus controls in two of six controlled-environment studies done. In my soybean controlled-environment study,

plants inoculated with lower levels of *B. japonicum* has 24% greater specific nitrogenase activity than that found for plants inoculated with higher levels of *B. japonicum*. In fact, plants inoculated with higher levels of *B. japonicum* and UW85 (HR+B) had lower specific nitrogenase activity levels than plants inoculated with lower levels of *B. japonicum* with or without UW85 (LR+B and LR-B). UW85 played no role in enhancing specific nitrogenase activity. Instead, in plants with lower root nodule DM and fewer root nodules, nodules were more active on a per gram basis. This inverse relationship between specific nitrogenase activity and nodule DM is a common observation in the literature (e.g. Burns et al. 1981; Chanway et al. 1989). Inoculation level of *B. japonicum* was the factor which restricted root nodule number and total nodule dry matter yield. Interestingly, no differences among treatments were found for nitrogen fixation efficiency (total mg plant N mg^{-1} nodule DM).

Previous to this thesis, the effects of UW85 on nitrogenase activity and root nodule number with species other than soybean had not been documented. In the controlled-environment study on field pea with a 17°C day/12°C night temperature regime, root nodule number per plant was 34% greater for plants inoculated with lower levels of *R. leguminosarum* bv. *viceae* and UW85 (LR+B) compared to those inoculated with lower rates of *R. leguminosarum* bv. *viceae* alone (LR-B). Unlike the previous discussion with soybean where increases in root nodule number were accompanied by increases in root DM, no differences among treatments for root DM were found. However, the difference in root nodule number between LR+B and LR-B treatments was not accompanied by any differences in specific root nodulation, specific nitrogenase

activity or nitrogen fixation efficiency. LR-B treated plants had root nodules with greater individual DM than LR+B treated plants. The larger individual nodule mass translated into total nodule DM that was not different from LR+B treated plants. LR+B and LR-B plants did not differ in total plant N content meaning that their equivalent nodule masses fixed the same amount of nitrogen over the same time period. This type of relationship is a common observation in the literature (e.g. Burns et al. 1981; Polonenko et al. 1987).

Previous research suggests that enhancements in soybean nitrogenase activity because of the use of UW85 occur (Halverson and Handelsman 1991; Halverson 1991). In this thesis, direct measures of nitrogenase acitivity did not support the idea. However, UW85 inoculated soybeans demonstrated promotions in plant tissue N content. Also, enhancements in soybean root nodule numbers documented in past work were seen again in this thesis along with limited evidence that such enhancements can occur in species other than soybean (i.e. field pea at the 17°C day/12°C night temperature regime) . Others have also documented increases in root nodule number due to PGPR use. In soybean, Singh and Subba Rao (1979), Burns et al. (1981), Polonenko et al. (1987), and Li and Alexander (1988) have documented increased root nodule numbers due to PGPR. As well this phenomenon has been demonstrated with a host of other plant species including peanut (Raverkar and Konde 1988), garden pea (Sarig et al. 1986), clover (Plazinski and Rolfe 1985a), common bean (Grimes and Mount 1984), chickpea (Alagawadi and Gaur 1988), alfalfa (Knight and Langston-Unkefer 1988) and lentil (Chanway et al. 1989).

Although the above studies have demonstrated PGPR-mediated increases in nodulation, measurements of nitrogenase activity have given mixed results. Burns et al.

(1988) have noted that when the use of *Azotobacter vinelandii* resulted in increased nodule numbers in soybean, nodules were smaller in size and no differences in nitrogenase activity per plant were found. Plazinski and Rolfe (1985a) stated that the extra nodules produced in their experiments with several *Azospirillum* sp. applied to clover were inactive when assayed for nitrogenase activity on a per plant basis. On the other hand, Yahalom et al. (1987), Algawadi and Gaur (1988) and Derylo and Skorupska (1993) documented research where both increases in root nodule number and nitrogenase activity per plant have occurred. Knight and Langston-Unkefer (1988) reported an experiment with clover where increases in nodule number and specific nitrogenase activity occurred. Derylo and Skorupska (1993), also working with clover, noted that they observed no increases in specific nitrogenase activity in their experiments although increases on a per plant basis did occur. Chanway et al. (1989) reported a series of indoor experiments with lentil where increases in root nodule number and/or nitrogenase activity per plant seemed to be most dependant on the planting medium.

In previous field research done with soybean and UW85 (Halverson and Handelsman 1991; Halverson 1991), increases in root nodule number were found at three experimental sites over three years but field measures of nitrogenase activity were not taken. In my thesis research, the inoculation of soybean with UW85 had no effect on nitrogenase activity per plant at 60 DAP at either the Winnipeg or Carman experimental sites. *B. japonicum* inoculation resulted in dramatic increases in nitrogenase activity and it was observed that soybean not inoculated with *B. japonicum* had no root nodules. For nitrogenase activity, results from the controlled-environment study done with soybean

were similar to the results found in the field.

No UW85-mediated differences among treatments in nitrogenase activity at 60 DAP were found for common bean, field pea or lentil grown at either experimental site. The effect of UW85 on nitrogenase activity levels in the field for species other than soybean has not been previously documented.

Increases in root nodule numbers of various species due to the use of PGPR's other than UW85 in field experiments have been noted by several authors including Raverkar and Konde (1988), Grimes and Mount (1984), Chanway et al. (1989) and Turner and Backman (1991). Sarig et al. (1986) found increases in nitrogenase activity per plant in clover and vetch although increased root nodule numbers were not found. Chanway et al. (1989) found that certain *Pseudomonas* species could influence the root nodule number or nitrogenase activity of Eston lentil depending on which strain of PGPR was used.

5.5 UW85 and Plant Growth Promotion - A Summary

At this point, the thesis hypotheses must be revisited. It has been shown that the inoculation of soybean with UW85 has resulted in promotions in the growth and yield of plants as compared to controls both in the growth chamber and in the field. It has also been shown that application of UW85 to soybean has resulted in increased plant tissue N content and root nodule numbers. Nitrogenase activity measured both in controlled-environment studies and in the field however, has been shown to not be promoted by use of the test strain. The hypotheses were originally made in reference to four test species including soybean, common bean, field pea and lentil. Lentil did not demonstrate any UW85-mediated promotions in the parameters outlined in the hypotheses. Common bean and field pea demonstrated some limited responses, both positive and negative, to UW85 use. In fact, decreased seedling survival and readily apparent symptoms of damage were found in controlled-environment work with common bean. In reference to the central concept outlined at the beginning of this discussion, it has been clearly shown that UW85 did have a positive effect on plant growth. However, these positive effects were species dependent. As well, UW85 had some negative effects on plant growth which were not expected. Previous to this thesis, only two instances of decreased seed yield in soybean due to UW85 use (Osburn et al. 1995) had been documented. In this thesis, instances of decreased plant growth (i.e. field pea), decreased N concentration and N content (i.e. field pea and soybean) and decreased seedling survival accompanied by visually apparent symptoms of damage (i.e. common bean) have been reported. While these phenomena occurred in controlled-environment studies only, they bring to light an

issue not formerly discussed with UW85, that being that this PGPR could also have a pathogenic side.

5.6 UW85 and Plant Growth Promotion - Possible Modes of Action

Several controlled-environment studies have shown that UW85 is a bio-control for *Phytophthora, Pythium, Sclerotinia* and *Aphanomyces* species (Handelsman et al. 1988; Handelsman et al. 1990; Handelsman et al. 1991; Phipps 1992; Smith et al. 1993; Silo-Suh et al. 1994). However, work has been done in gnotobiotic controlled environments which demonstrates plant growth promotions in the absence of disease (Halverson and Handelsman 1991; Handelsman 1991). Halverson and Handelsman (1991) stated that, "nodulation enhancement does not depend on the suppression of *Phytophthora* or *Pythium* disease." They further stated that, "UW85 likely affects the nodulation process soon after planting by stimulating bradyrhizobial infections or by suppressing the abortion of infections." They speculated that enhancements of nodulation by UW85 would change the nitrogen status of inoculated soybean and so promote plant growth.

The controlled-environment studies included in this thesis were also conducted under gnotobiotic conditions. Soybean inoculated with higher rates of *B. japonicum* and UW85 (HR+B) had greater numbers of root nodules than plants inoculated with higher rates of *B. japonicum* alone (HR-B). This supported results found by Halverson and Handelsman (1991) and Handelsman (1991). However, since both lower (LR) and higher (HR) inoculation rates of *B. japonicum* were used, other inferences about UW85's mode of action can be made. No increases in root nodule number were found for soybean inoculated with lower rates of *B. japonicum* and UW85 (LR+B) versus soybean inoculated with lower rates of *B. japonicum* alone (LR-B). Higher concentrations of

B. japonicum present in the rhizosphere was an important pre-condition for UW85 use to result in increased root nodule numbers. Root growth can be promoted but sufficient symbionts must be present to take advantage of a greater number of potential infection sites.

No differences among treatments were found for specific root nodulation in my soybean controlled-environment study. Increases in nodule number corresponded to increases in root DM for soybean inoculated with higher rates of *B. japonicum*. Plants inoculated with lower rates of *B. japonicum* behaved differently. When UW85 inoculation accompanied inoculation with a lower rates of *B. japonicum* (LR+B), root mass was greater than that found for plants inoculated with lower rates of *B. japonicum* alone (LR-B) and was similar to that found for control plants and plants inoculated with higher rates of *B. japonicum* alone (HR-B). However, no increase in root nodule number was found for LR+B treated soybean compared to LR-B treated soybean. In past work, growth promotions due to UW85 have been attributed to increases in nodulation (Halverson and Handelsman 1991; Handelsman 1991; Osburn et al. 1995). The above evidence suggests UW85 can cause growth promotions through means other than increasing nodulation (i.e. UW85 stimulating root growth).

Previous work with UW85 has also attributed soybean growth promotions to increased nitrogenase activity levels (Halverson and Handelsman 1991; Handelsman 1991; Osburn et al. 1995). In my soybean controlled-environment study, nitrogenase activity levels were found to be unaffected by UW85 use on a per gram nodule basis (specific nitrogenase activity). It should be noted that root N concentration for plants

inoculated with UW85 was found to be lower than for those without such inoculation. UW85-mediated promotions in whole plant and nodule N content of HR+B treated soybean versus HR-B treated soybean were found. N fixation efficiencies did not differ among treatments. In my soybean controlled-environment study, UW85 use did not result in promotions of nitrogenase activity but such use did result in increases in the amount of N fixed by plants as reflected by measures of N content.

Observations from soybean grown at the Winnipeg field site provide further evidence to show that stimulation of nodulation and nitrogenase activity by UW85 use alone is not a satisfactory explanation of the mode of action. Bearing in mind that soybean not inoculated with *B. japonicum* were noted as having no nodules on their roots at the Winnipeg site, it is significant that plants inoculated with only UW85 (-R+B) had greater seed and pod N concentration, whole shoot N content and stem and leaf N content than plants not inoculated with UW85 or *B. japonicum* (-R-B) at 90 DAP. At 120 DAP, the seed DM yield of -R+B treated plants was 23% greater than that found for -R-B treated plants. Growth promotions with soybean in the field did occur in the absence of nodules and N_2 fixation.

Turner and Backman (1991), working with a *Bacillus subtilis* strain, discovered that their strain stimulated the growth of peanut roots under moisture stressed conditions. They also found that along with increased N content, treated plants also had increased potassium and boron content. Turner and Backman (1991) noted that, while they first thought their PGPR was enhancing nodulation, further evidence led them to believe that root growth was being stimulated and that any effects on nodulation or increased nutrient

uptake was a result of increased root growth. In my research, I have provided evidence to show that increases in nodulation and nitrogen fixation are not adequate explanations for UW85-mediated promotions in soybean growth, yield and N content. UW85 may be promoting the growth of soybean roots. I did find UW85-mediated root DM increases in my soybean controlled-environment study while increases in specific root nodulation were not found. Halverson and Handelsman (1991) and Halverson (1991) reasoned that, since they detected increased nodulation within 21 to 28 DAP in their field experiments, UW85 must have been affecting the process of nodule formation at 10 to 12 DAP. However, Halverson (1991) suggested that UW85-mediated promotions in soybean root length and shoot height up to 49 DAP found in one of their field experiments could be explained by UW85 reducing disease pressure, improving phosphorus nutrition or enhancing components of the rhizosphere microbial community that promote plant growth.

My research with other species provides evidence that UW85's mode of action may vary depending on the species it is applied to. In my controlled-environment study with common bean, UW85 did not cause any growth promotions and caused seedling damage. In my controlled-environment study with field pea grown in a warmer temperature regime (22°C day/ 17°C night), UW85 use resulted in reductions in growth when lower doses of *R. leguminosarum* bv. *viceae* were used. In my controlled-environment study with field pea grown in a colder temperature regime (17°C day/ 12°C night), UW85 use resulted in increased root nodule numbers when lower doses of *R. leguminosarum* bv. *viceae* were used. However, individual root nodules were larger

when UW85 was not used. With all of these species no differences among treatments for specific nitrogenase activity or nitrogen fixation efficiency were found. In the field, no growth promotions due to UW85 were demonstrated with common bean, field pea or lentil.

5.7 Potential of UW85 in Agriculture

Because of a shift in focus towards issues of pollution, food safety and the use of non-renewable resources in conventional agriculture, PGPR have become an active area of research interest (Jacobsen and Backman 1993; Glick 1995). Jacobson and Backman (1993) noted that increasing numbers of producers are entering the organic market and that they view biological products as an alternative to synthetic inputs. The implication is that, besides conventional agriculture, organic production is a ready and growing market for PGPR-type products. PGPR type products already exist in the market place (Okon 1985; Okon and Hadar 1987; Turner and Bachman 1991; Mahaffee and Bachman 1993). As well, UW85 has been screened in several potential commercial formulations (Osburn et al. 1995).

There has been ample discussion about the commercial applications of PGPR. Suslow (1982) suggested that the use of PGPR on potato and sugar beets would be a good economic risk assuming an average yield benefit of 10%. However, he cautioned that cost-benefit realities would require extensive testing of PGPR under a range of conditions. Reviewing work on *Azospirillum* sp., Okon (1985) felt that there would be commercial applications in both intensive and extensive agriculture. Reduced fertilizer use, increased production on fully fertilized fields and increased production in semi-arid areas were all cited as the benefits from *Azospirillum* sp. use. However, Okon (1985) stressed that any successful, widely used products must produce consistent effects on yield. When considering commercial PGPR products it is not unreasonable to suggest that potential consumers of these products would look at yield increases of marketable

plant parts as a measure of how useful the products are. The need for PGPR to provide consistent increases in yield has been identified as a central problem by many authors (Broadbent et al. 1977; Suslow 1982; Schroth and Hancock 1982; Burr and Caesar 1984; Okon 1985; Schippers et al.1986; Kloepper et al. 1989; Elsas and Heijnen 1990; Mahaffee and Backman 1991; Kapulnik 1991; Jacobson and Backman 1993). Suslow (1982) documented that 32 of 69 trials on potato and sugarbeet conducted from 1978 to 1982 demonstrated significant increases in yield due to PGPR use. These increases ranged from 6 to 33%. Kloepper et al. (1989) in a similar effort noted that in 22 studies on PGPR from 1974 to 1989 covering 11 crop species, the percent yield difference from the control ranged from -17% to +160%. In 65% of these studies, PGPR use resulted in yield decreases as well as yield increases.

While inconsistent performance is a problem with PGPR, some research shows promise. Of note is work by Turner and Backman (1991) who examined the performance of *Bacillus subtilis* strain A-13 with peanut in regional field trials done in Georgia, Alabama and Florida in co-operation with 24 producers. The average yield increase due to use of their strain across all locations was 7.6% with a response range of -3.5 to +37%. Only 2 locations demonstrated negative responses to PGPR use. In 16 locations, planting date and crop rotation histories were available. The authors found that locations which had been planted early and had legumes in the previous two years of their rotational histories, demonstrated greater yield increases than locations which were planted later or had no legumes as previous crops. No differences among treatments in disease severity during the growing season were noted. This work is significant because it is one of the

few instances in the literature where a PGPR was tested in commercial production systems over several locations. As well, this work is unique in that an attempt was made to account for inconsistent performance based on documented agronomic practice differences between sites. Kloepper et al. (1989) stated that yield effects in field trials are inconsistent due to the complexity of the system which determines yield. Turner and Backman (1991) made an attempt to account for some of this complexity.

Past field research with UW85 has demonstrated frequent promotions in yield at P=0.10 (Halverson 1991; Osburn et al. 1995). However, at P=0.05 no instances of yield promotions were noted by Halverson (1991) and, with Osburn et al. (1995), yield promotions attributed to UW85 use dropped by 133%. Kloepper et al. (1989), in discussing the analysis of PGPR field experiment yield data, emphasized that effects are real if the mean difference of a treatment is significant at P=0.05 or lower. They also emphasized that evaluating product performance inconsistency and attempting to correct for this problem should be central themes in PGPR product development. In past work with UW85 (Halverson and Handelsman 1991; Halverson 1991; Osburn et al. 1995) there has been a tendency to document yield differences at P=0.10. Using a greater P value is not an answer to problems with inconsistent performance. Our research with UW85 further documents inconsistent performance in the field. Yield promotions were dependent on location (Winnipeg versus Carman), plant species and *B. japonicum* inoculation status (with soybean). One possible explanation for the differing performance between the Winnipeg and Carman sites in relation to soybean involves soil moisture. In the month of June, the Carman site received substantially less rainfall than the Winnipeg

site or than the long-term normal for the Carman area (Table 3.1) . The high rainfall in Winnipeg could have been conducive to the survival and proliferation of UW85 since it is a facultative anaerobe (Handelsman et al. 1990). As well, UW85 at the Carman site could have produced endospores and become dormant in June because of the low rainfall conditions. This would have limited UW85's ability to survive and grow at this site. Therefore, differences in performance between sites could have been due to differences in UW85 survival and proliferation in plant rhizospheres as influenced by soil moisture and rainfall amounts. Another point to note is that seed yield and plant part DM for soybean were greater at Carman compared to Winnipeg. The higher rainfall amounts at Winnipeg could have led to denitrification and a lowering of soil N content. If UW85 does promote the growth of roots, it is in such a situation that a benefit might be seen. If the roots of UW85-treated soybeans were larger than untreated soybeans, they would have explored more soil and accessed more N with the possible result being increased plant growth. At Carman, higher soil N levels may have made larger root size and the ability to explore more soil less advantageous. Whatever the reasons, consistent performance is still a major hurdle for UW85.

Many who have worked with *Bacillus* species including UW85 (Handelsman et al. 1990; Turner and Backman 1991; Liu and Sinclair 1993; Silo-Suh et al. 1994; Osburn et al. 1995), have noted characteristics of the genera which facilitate commercialization. These include Bacilli being facultative anaerobes, being able to form highly stable compounds which are biologically active and being able to sporulate. Of these characteristics the last is cited most often because it is felt that endospore formation

facilitates strain survival within an inoculum formulation resulting in products that are durable and consistent in quality. This is expected to translate into more consistent field performance. As well, endospores can present processing advantages in certain types of formulations (Turner and Backman 1991). It is interesting to note that there has been virtually no discussion of human health risks associated with *Bacillus* species including *B. cereus* in the literature pertaining to PGPR. *B. cereus* strains have been noted as the cause of *B. cereus* food poisoning (Claus and Berkeley 1986). In my research, UW85 was field tested in a granular formulation and did cause several different promotions including increases in soybean seed yield. Further, this granular product was similar, in terms of handling and metering, to several other types of granular products commonly used by producers. Unique and perhaps costly equipment would not be needed, thus addressing concerns raised by Jacobson and Backman (1993) and Hagedorn (1993) about poor product adoption due to costly equipment or time requirements. Although problems with consistency of performance exist, "commercial-like" formulations of UW85 have been tested successfully.

5.8 Future Research with UW85

To date research on UW85 has focused most often on its bio-control potential. There is no doubt that the strain has a future in this realm. UW85's potential as a PGPR has only been documented on three occasions prior to this thesis (Halverson and Handelsman 1991; Halverson 1991; Osburn et al. 1995). Several areas of investigation remain open.

First, UW85 has to be tested as a PGPR with more species than it has in the past. My work has shown that UW85 can have variable effects depending on what species it is applied to. Mahafee and Backman (1993) note, in reference to their work with a *Bacillus subtilis* strain and cotton, that cultivar may be a factor in the effectiveness of a PGPR. At this point, UW85 has been shown to be effective, although not consistently, with five different soybean cultivars under various conditions. Further testing with other soybean cultivars should be done.

My work has shown that growth promotions can take place with soybean in the field when no nodules have been formed. Past speculation about UW85's mode of action has suggested direct effects on either the plant or the symbiont that enhance nodulation and N fixation (Halverson and Handelsman 1991; Handelsman 1991). Work like that of Turner and Backman (1991) where root growth promotions were documented using a rhizotron could be important in determining UW85's mode of action. Controlled-environment studies done under gnobiotic conditions are also important in trying to sort out the possibilities. Legumes should be tested without the appropriate symbiont present. Such tests are very effective in determining if the processes of nodulation and N_2 fixation

are being directly affected by UW85.

In future work with UW85 it will be important that nutrient concentration and content of plant tissues be determined. This has implications for determining UW85's mode of action and also has implications for UW85's commercial potential. Besides yield, enhancements in the quality of that yield could prove to be an important selling point. In future work, the plant tissue concentration and content of other nutrients should also be documented.

If UW85 is developed as a product, it will be important that agronomic factors be taken into account in a manner similar to that reported by Turner and Backman (1991). This work allowed Turner and Backman (1991) to develop criteria for determining the future success of their strain. Such work could allow a manufacturer to develop a product which offers consistent performance because it is used in situations where it is most likely to work.

It is important that determining UW85's mode of action becomes a significant focus in future research. Research has already identified antibiotics produced by UW85 which protect several species from diseases (Handelsman el al. 1988; Silo-Suh et al. 1994). The development of UW85 as a PGPR would benefit greatly from a similar level of understanding.

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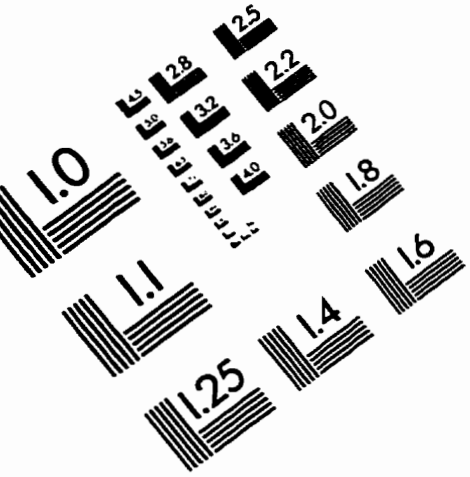
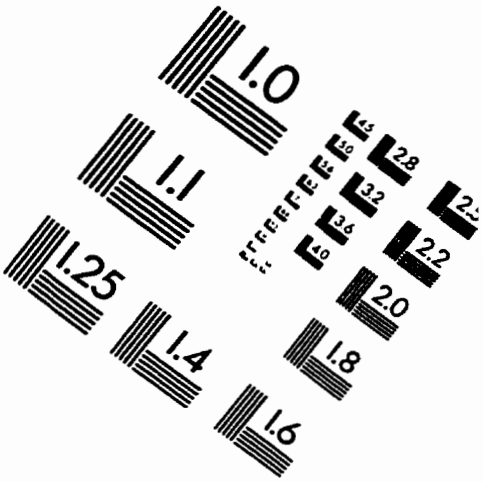
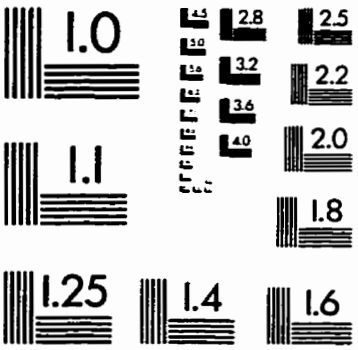
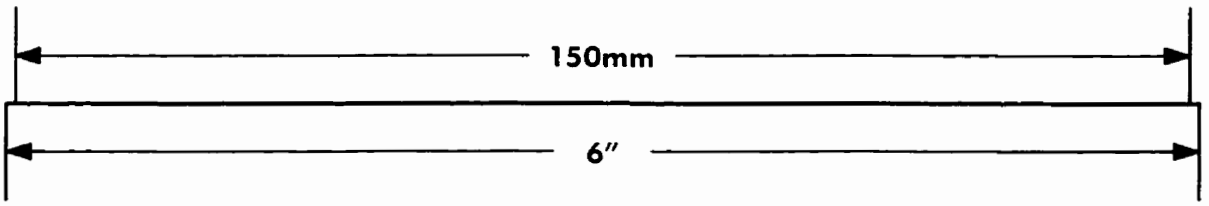
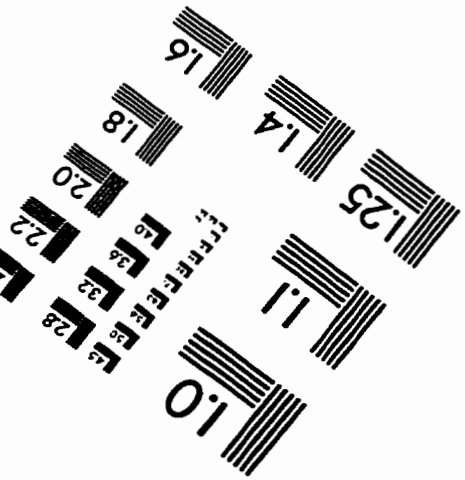
IMAGE EVALUATION TEST TARGET (QA-3)

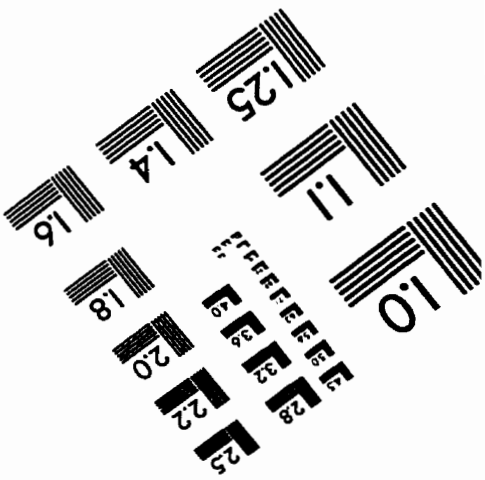












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