ASPECTS OF THE PHYSIOLOGY AND AGRONOMY OF COMPETITION IN CROP PLANTS

by

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Short Title

PHYSIOLOGICAL ASPECTS OF COMPETITION IN CORN POLYCULTURE SYSTEMS

Omar A.K. Abdin

This thesis is dedicated to those to whom I am eternally indebted for their support, understanding, and constant encouragement throughout my period of study.

To my parents, wife and my son Ali.

Abstract

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ASPECTS OF THE PHYSIOLOGY AND AGRONOMY OF COMPETITION IN CROP PLANTS

The overall objective of this research was to identify forage legumes and grasses, which competed sufficiently with weeds developing after tillage, to provide acceptable weed control in grain corn, without, however reducing corn growth, and to obtain a better understanding of the physiological aspects of competition, particularly light and nitrogen. Corn yields were not affected by forage seeding date (10 and 20 days after corn emergence), however yields were affected by the type of forage species used as an intercrop. Cultivation reduced weed populations in corn and the inclusion of cover crops provided some additional control (up to 10%). Crimson clover was able to establish and suppress weeds well, however, it caused yield reductions in grain corn. A system of injecting solutions into stems of soybean plants proved to be successful for administering substantial amounts of dilute and concentrated solutions over long periods of time. Injection of exogenous sucrose caused increased dry matter accumulation in soybean plants. The injection of sucrose plus nitrogen, resulted in increased plant dry weight, grain yield, and delayed senescence, but only under unshaded or slightly shaded conditions. Highly shaded plants were not affected by the injection of sucrose or nitrogen, suggesting other mechanisms, that are light dependent, control dry matter production and plant senescence at lower light intensities. Plants placed under 75% shade senesced approximately two weeks earlier than unshaded plants. Soybean plants injected with sucrose or sucrose plus nitrogen senesced later than those receiving only nitrogen or distilled water, demonstrating that carbon availability plays a more important role than nitrogen in the senescence of soybean plants.

RESUME

LES ASPECTS PHYSIOLOGIQUES ET AGRONOMIQUES DE LA COMPETITION ENTRE LES CULTURES.

L'objectif de cette recherche était d'identifier l'espèce fourragère qui peut achever un bon contrôle des mauvaises herbes qui se développent après un sarclage, sans pour autant faire la compétition au maïs-grain, et de mieux comprendre l'aspect physiologique de la compétition surtout pour la lumière et l'azote. Le rendement du maïs-grain a été affecté par les espèces fourragères intercalaires et non par leurs dates de semis (10 et 20 jours après l'émergence du maïs). Le sarclage a réduit les populations des mauvaises herbes et les intercalaires ont fourni un contrôle supplémentaire (jusqu'à 10%). Le trèfle incarnat s'est bien établi et a bien contrôlé les mauvaises herbes, cependant il a causé des réductions du rendement du maïs. Un système d'injection de solutions dans les tiges des plants de soja a prouvé être efficace pour administrer aux plantes différentes quantités de solutés pendant de longues durées. L'injection de sucrose exogène a causé une augmentation de l'accumulation de la matière sèche dans les plants de soja. L'injection de sucrose et d'azote en même temps a résulté en une augmentation du poids sec des plantes et de leur rendement, un retard de la sénescence mais seulement dans des conditions ombragées ou semi-ombragées. Les plants fortement ombragés n'étaient pas affectés par l'injection de sucrose et d'N. Ceci suggère que d'autres mécanismes, qui dépendent de la lumière, contrôlent la production de la matière sèche et la sénescence sous de faibles intensités lumineuses. Les plants placés dans des conditions de 75% d'ombre ont sénescé à peu près deux semaines plus tôt que ceux qui ne l'étaient pas.

Les plants recevant du sucrose ou du sucrose + N ont sénescé plus tard que ceux qui ont reçu de l'N seulement ou de l'eau distillée. Ceci démontre que la disponibilité du C joue un rôle plus important dans la sénescence des plants de soja que l'N.

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CONTRIBUTIONS OF CO-AUTHORS TO MANUSCRIPTS FOR PUBLICATION

The contents of chapter 3 were drawn from a manuscript that has been submitted for publication in the Agronomy Journal. The contents of sections 4, 5, 6, 7, and 8 are to be submitted for publication. All the manuscripts were, or are to be, co-authored by myself O.A. Abdin, my supervisor Dr. Donald L. Smith, Dr. B.E. Coulman, Dr. Mohammed A. Faris, and Dr. Daniel Cloutier.

Dr. Daniel Cloutier provided expertise on the competitive aspects of weeds, aided in supervision of the field work and provided me with the necessary field facilities at the Agriculture and Agri-Foods Canada research station at l'Assomption. He also carefully reviewed my manuscripts and provided me with reference material that I was unable to collect on my own.

Dr. Bruce Coulman aided in the design of the field experiments, provided expertise on forages, provided me with supervision during my first year of study, and reviewed my manuscripts for publication.

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Dr. Donald Smith has provided guidance in the development and utilization of the injection technique, access to field and greenhouse space, computers and laboratory instruments at the Macdonald Campus of McGill University and funds to support the research. He also reviewed all the manuscripts and other sections of this thesis.

Chapter 2

GENERAL INTRODUCTION

2.0. Cover crops:

Use of cover crops in cropping systems dates as early as 400 B.C.. According to Semple (1928), several writers of ancient Greece have studied and compared the effectiveness of different leguminous cover crops and discussed their effectiveness in soil improvement. Pieters (1927) reported that Chinese writers noted an increase in the yield of crops following a legume winter cover crop. With the decline in soil fertility across the centuries, the importance of legume cover crops should have become more apparent. But according to Smith et al. (1987), this problem was probably avoided by long term pasture rotations, and application of animal manure to grain crops. Active research on the importance and value adding of cover crops in agriculture commenced during the first half of this century. A decline in the hectarage of green manure crops was noticed in the 1940's. According to Smith et al. (1987), this decline was mainly due to the widespread availability of synthetic nitrogen fertilizers, and the economic benefits to farmers from continuous grain crop production, so that the practice of growing winter cover crops has become increasingly limited.

During the past decade there has been renewed interest in growing cover crops. Smith et al. (1987) suggested that this has been due to three main reasons. First, an increasing in the price of synthetic fertilizers, which is anticipated to be more expensive in the future and eventually in limited supply. Second there was an increase in concern about soil erosion. Lastly, there has been was an increased adoption of no till systems.

2.1. Beneficial effects of cover crops:

2.1.1. Soil organic matter and physical properties:

One of the most important benefits of cover crops is that they provide an increase in soil organic matter through the addition of plant material to the soil. Gosdin et al. (1949) found an increase in soil organic matter when corn was interseeded with legumes. Muzurak and Conard (1966) found that alfalfa increased soil organic matter as compared with grass or a grain-fallow system after a period of seven years. Hargrove (1986) reported that soil organic carbon and nitrogen either remained constant or declined only slightly after three years in no till sorghum with several cover crops in comparison with a winter fallow treatment. Millhollon et al. (1994) reported results from a 35-year experiment which showed that soil organic matter content in plots seeded with hairy vetch increased significantly in comparison with winter fallow plots. Plenet et al. (1993) incorporated a rye grass winter cover crop with maize rotation and reported a 2-3 t ha^{-1} increase in the amount of soil stored carbon after 22 years. Sur et al. (1993) reported that green manure caused an increase of 25 to 50% in soil organic matter.

Organic matter plays an important role in improving the physical properties of the soil. Ram and Zwerman (1960) found that soil aggregate stability was improved by 30% or more due to the addition of a rye grass green manure in a silty clay loam for seven years. Morachan et al. (1972) reported a decrease in bulk density and an increase in total soil porosity to be associated with the addition of green manure for 13 consecutive years. Reid and Goss (1981) reported that root growth of perennial rye grass (*Lolium multiflorum*) and of lucerne for 42 days was generally associated with an increase in aggregate stability; this effect was probably caused by organic substances released from the roots which either stabilize the aggregate directly or indirectly after microbial attack. Tisdall and Oades (1982) found that stability of soil aggregates increased when soil organic matter increased following incorporation of plant residues. Sur et al. (1993) have reported that green manuring decreased the bulk density of soil, and increased soil aggregate stability, porosity, and water retention. Gerzabek et al. (1995) showed that over a 38-year period there was an increase in soil aggregate stability in soils in which green manure was incoporated over bare fallow soil. Breland (1995) reported that undersowing ryegrass and white clover (*Trifolium repens*) in a cereal crop tended to improve soil aggregate stability, bulk density and porosity the following season.

2.1.2. Soil erosion:

Smith et al. (1987) pointed out the benefits of the use of cover crops as a method of reducing soil erosion, and suggested three mechanisms through which cover crops can reduce runoff and erosion. First, the increase in soil porosity due to cover crops causes water to infiltrate into the soil rather than running off the surface. Second, the increase in water transpiration by cover crops stimulates evaporation which depletes soil moisture causing water to infiltrate into the soil rather than runoff. Third, the standing cover crop tends to reduce the energy of the rain drops falling on the soil, decreasing the splash effect, and reducing the velocity of the water moving over the surface of the soil. Mati (1994) reported an exponential decrease in the amount of splashed soil moving down a slope with the increase in cover crop. Frye et al. (1985) reported that losses from conventional tillage were twice the tolerated limit, whereas inclusion of a winter cover crop in combination with no tillage reduced soil losses by 2.0 Mg ha^{-1} yr^{-1}. Estler and Hargrove (1991) reported that including cover crops between corn rows during the growing season decreased soil erosion by 2.4% compared with conventional methods. Edwards et al. (1993) reported a decrease in soil loss to about 10% below the tolerance limit when a rye (*Secale cereale*) cover crop was included in a rotation during the winter season. Average soil loss before the winter cover treatment was approximately ten times greater than after incorporating the cover crop. Soileau et al. (1994) found that incorporating rye as a winter cover crop with conservation tillage reduced sediment loss by approximately 50%.

2.1.3. Nitrogen production :

One of the benefits of leguminous cover crops is the fixation of atmospheric nitrogen into plant available forms which decreases the overall dependence on synthetic nitrogen fertilizers. This reduces the energy use and pollution potential associated with crop roduction. Nitrogen contribution to the soil is mainly due to nitrogen fixation. Heichel (1985) noted that the amount of nitrogen contributed by the legumes depends on the symbiotic nitrogen fixation activity, the amount and type of residues left in the soil, and the availability of soil nitrogen to the legume. Heichel and Barnes (1984) pointed out that the crowns and roots of forage legumes harvested for hay are the main source of nitrogen to the soil in a hay production system. In contrast, Groya and Sheafer (1985) found that 17, 27, 37, and 18% of the total plant nitrogen had accumulated in the crowns and roots of non-dormant (annual) and dormant alfalfa, red clover and sweet clover, respectively, when harvested for hay.

Estimates of nitrogen contribution by alfalfa to the following crop have been as high as 180 kg N ha^{-1} (Baldock and Musgrave, 1980; Voss and Shrader, 1984). Jones et al. (1977)

reported that over a three-year period, subclover (*Trifolium subterraneum*) fixed 261, 398, and 207 kg N ha^{-1} respectively as estimated by the difference method. Heichel et al. (1981) reported that the average nitrogen contribution in the establishment year of an alfalfa crop was 148 kg N ha^{-1} and that nitrogen fixation contributed 43% of that amount. Ebelhar et al. (1984) estimated that hairy vetch provided the equivelant of about 95 kg of fertilizer nitrogen ha^{-1} to corn. Kelner and Vessey (1995) reported that the alfalfa cultivar Nitro added an average of 12.4 kg ha^{-1} of fixed nitrogen to the soil in the autumn. Papastylianou (1993) reported that the total amount of fixed nitrogen in common vetch (*Vicia sativa*) was approximately 180 kg N ha-1, and that 58-65% was estimated to have remained in the roots after harvesting for hay. In another study by Stute and Posner (1995), they have reported that nitrogen released from hairy vetch and red clover resulted in mineral nitrogen levels in the soil similar to those following an application of 179 kg ha^{-1} of fertilizer N. Moreover, residues decomposed rapidly releasing half their nitrogen within 4 weeks after burial.

Bolger et al. (1995) reported that the total amount of fixed nitrogen accumulated in the shoot biomass of subterranean clover (*Trifolium subterraneum* L.) ranged from 50 to 125 kg ha^{-1}.

2.2. Interseeding cover crops in corn:

2.2.1. Effect on corn yield:

Several studies have shown that seeding forages at the same time as corn would cause substantial yield reductions (Schaller and Larson, 1955; Ampong, 1985). Nordquist and Wicks (1974) reported that when alfalfa was simultaneously sown with corn, grain yields were reduced by 1 to 3 t ha^{-1} when compared with alfalfa planted at last cultivation. Scott et al. (1987), delayed forage seeding until corn was 15 to 30 cm in height and reported no grain yield reduction. Claude et al. (1993) reported a corn yield reduction when red clover was interseeded with corn, and that much of this reduction was due to the inability of red clover to compete with the existing weeds. Tomar et al. (1988) reported a 2 t ha^{-1} reduction in corn yield corn was intercropped with red clover in 5 site years, however yield was not affected in one site in which rainfall was abundant.

On the other hand, Pleasant et al. (1991) reported no reduction in corn grain yield when corn was interseeded with red clover (*Trifolium pratense*) or ryegrass (*Lolium spp.*). Stemann et al. (1993) seeded forage grasses under corn at seeding, at emergence, at the 2 to 3 leaf, and 6 to 8 leaf stages. Silage yields were not affected when grasses were interseeded at the 2 to 3 leaf stage. Corn yields were slightly increased when corn was 30 cm high.

Exner and Cruise (1993) interseeded an equal mixture of an alfalfa, sweet clover (*Melilotus officinalis*), red clover, and white clover (*Trifolium repens*) between corn rows at the time of corn seeding and at the last cultivation. They reported that corn yields were not reduced when the forages were sown late, but were reduced when seeded at the early date because weed control by interrow tillage was interrupted. Moureaux et al. (1992) interseeded corn with several forages (perennial ryegrass, red clover, alfalfa, and winter rye) and reported no reduction in corn grain yields. Wall et al. (1991) intercropped silage corn with red clover and reported no effect on the corn yield. Similar results were reported by Warman (1990) who found that intercropped red clover had no effect on corn yields and did not compete with the corn for available nutrients.

2.2.2. Effect of legumes on succeeding crop yield:

The value of legumes in crop rotation has long been recognised. Nitrogen fixed by legumes is contributed to succeeding non-fixing crops upon decomposition of legume top and root material (Bruulsema and Christie, 1987), therefore increasing the yield of the succeeding crop (Fribourg and Johnson 1955; Bartholomew, 1965).

Many researchers have reported that grain yields of non-legume crops are increased by a previous legume crop (Baldock et al., 1981; Hoyt and Leitch, 1983; Groya and Sheafer, 1985; Hargrove, 1986). Decker et al. (1994) reported that corn grain yield after legume cover crops was greater than after no cover crop. Similar results were reported by Bollero and Bullock (1994) where corn grain yield following rye (*Secale cereale*) was lower that following hairy vetch. Stoa and Zubrisky (1969) reported that wheat grain yields without nitrogen fertilization were nearly 50% higher from land previously cropped with alfalfa than from a non-legume rotation, and 10 to 15% higher yields following the legume when fertilized with 67 kg N/ha. Fertilizer nitrogen equivalence of one year of red clover for first year corn was the same as from a 1 year stand of alfalfa (Bruuselma and Christie 1987). Schmid et al. (1959) reported that the fertilizer nitrogen equivalence of a two-year stand of birds foot trefoil was similar to alfalfa for first year of corn, but less than for second year corn, they found no effect of alfalfa, red clover, and birds foot trefoil on the yield of succeeding crop. Legume nitrogen can contribute substantially to the nitrogen needs of succeeding crops in a rotation, but amounts available depend on the duration of growth of the legume species, the amount of biomass incorporated, the legume species grown, and the succeeding crop species grown.

Organic nitrogen in ploughed down forage legumes becomes available to subsequent crops (Hoyt and Leitch, 1983), but there may also be more nitrogen available for the succeeding crop due to less removal of soil nitrogen by legumes.

Seneratne and Hardson (1988) reported that nitrogen benefit to crops following grain legumes was due to lower uptake of soil nitrogen by legumes than cereals and a carry-over of nitrogen from the legume residue, both leading to greater uptake of soil nitrogen by the subsequent non-legume crops compared with crops grown after other non-legumes. Robinson (1968) found that corn grain yields following unharvested alfalfa were greater than those after harvested alfalfa. Meyer (1987) reported that barley grain yields following four to six hayed legumes were increased by 7 to 68% compared with barley following wheat without fertilization. Fertilized (75 kg N ha^{-1}) barley yields were 12 to 15% greater following the hayed legumes than following wheat. In another study by Meyer (1987), wheat grain yields were 96% higher following green manure sweet clover than continuous wheat, and 9% higher than following fallow when unfertilized, and 31% and 10% higher respectively, when fertilized with 56 Kg N ha^{-1}.

2.2.3. Effect on weed suppression:

It is commonly reported that intercropping can be an efficient means of weed control. Steiner (1984) observed that in many intercropping systems only one mechanised weeding was required to produce optimum yields instead of the two or three weedings required in mono crops. The living mulch can produce a low-growing, high-density cover that suppresses weeds between rows of taller desirable crop species such as maize and sorghum. Enache et al. (1990) reported that a subterranean clover living mulch effectively controlled ivy leaf morning glory (*Convolvulus* spp.).

In corn production studies in Pennsylvania, Hartwig (1976) found that a living mulch of crown vetch (*Cornilla varia* L.) can be competitive with weeds leading in this study to the suppression of yellow nutsedge (*Cyperus esculentus* L.). Varbel et al. (1980) evaluated various legume mulches in corn and found that white clover (*Trifolium*) effectively suppressed weeds while red clover was a poor competetor. Seeding living mulchs five weeks prior to corn seeding rather than five weeks after seeding provided better weed control but lower corn yield.

Shading of the soil and competition for water and nutrients suppresses weed germination and growth (Altieri and Liebman, 1986). Living mulches or cover crops produce such conditions thereby suppressing weed populations. Cover crop residues can also influence weed populations in no-till cropping systems because of the proximity of the residues to the site of seed germination on the soil surface. Mohler (1991) reported that the presence of a dead mulch of rye decreased weed biomass and had no detrimental effect on corn yield. Teasdale et al. (1991) reported that rye or hairy vetch residues reduced total weed density an average 78% compared with the same treatment without a cover crop when cover crop biomass exceeded 300 g m^{-2} and when residues covered more than 90% of the soil. Annual legumes (White et al., 1989) and rye (Shilling et al., 1986; Barnes et al., 1987) release allelopathic compounds that suppress germination and growth of some weed species. Samson (1991) reported that interseeding ryegrass with corn consistently reduced weed biomass by about 50%. A recent study by Mohler and Callaway (1995) reported that the presence of fall rye (*Secale cereale* L.) decreased the seed production of *Portulaca oleraceae* L. in one year.

De Haan et al. (1994) suggested that spring seeded smother crops can reduce weed populations by up to 80% with little effect on corn yield. Hoffman et al. (1993) reported that a hairy vetch (*Vicia villosa* Roth.) cover crop reduced weed biomass by 96% without causing any reduction in the corn yield when the vetch was planted in May or June. Teasdale (1993) reported that hairy vetch residues inhibited the establishment of common lamb's quarter (*Chenopodium album* L.) without affecting corn establishment. Other legumes, such as subterranean clover, have been reported to provide equal or better weed control in no till corn than herbicide treatments without a decrease in corn yields (Enache and Ilnicki, 1990; Ilnicki and Enache, 1992). Johnson et al. (1993) reported that a hairy vetch and rye cover provided good weed control in no till corn production. Palada et al. (1982) reported a 75% decrease in the number of weeds present when corn was interseeded with red clover or hairy vetch.

2.3. Competition between components of intercropping systems and weeds:

Competition among plants of different species as well as those of the same species involves many factors. McCloud and Mott (1953) stated that the performance of different species in mixtures varied from mutually depressive to no interaction to beneficial, illustrating the multiplicity of factors involved. Competition between neighbouring plants commonly occurs for three main environmental factors: light, nutrients, and water (Donald 1963; Rhodes 1970).

2.3.1. Light:

Competition for light may occur whenever one plant casts a shadow on another or when one leaf shades another (Donald 1963) and the successful plant is the plant which has its foliage in an advantageous position, relative to the foliage of its competitor (Etherington 1976). Chestnutt and Lowe (1970) reported that competition for light may also influence the rate of nitrogen transfer from legumes to grasses, as shading of the legumes restricts the supply of carbohydrate to the root system, thus causing death of nodule tissue and possibly an increase in the rate of nitrogen transfer to the competing grasses. Butler et al. (1959) and Mckee (1962) showed that rapid root nodule senescence due to shading occurs in white clover and BFT, but alfalfa and red clover are more tolerant to this treatment.

Harris and Thomas (1973) observed the initial dominance by ryegrass in a ryegrass/white clover pasture and attributed this inpart to relatively greater growth rate of rye grass under the low light and low temperature regimes of winter, resulting in the shading of clover. This shading was increased by increasing intervals between defoliation. Chamblee (1972) reported that alfalfa cultivars utilised in North America tend to dominate their companion grasses, due to more advantageous canopy structure of alfalfa which enables adequate light penetration to lower levels.

2.3.2. Nutrients:

Studies on interference between plant species have led to the conclusion that competition for nutrients can be of greater importance than competition for light (Snaydon, 1971; Eagles, 1972).

Most legumes compete poorly with grasses for nitrogen. Competition for nitrogen between grasses and legumes is influenced not only by the species involved, but also by the source of nitrogen. Walker et al. (1956) found that when grass and clover were grown together in a pot, the grass took up practically all the nitrogen (over 90%) and took up almost as much as it did when grown alone. In association with grasses, white clover is also a poor competitor for P (Mouat and Walker, 1959; Jackman and Mouat, 1972), K (Mouat and Walker, 1959) and S (Walker and Adams, 1958). The poor competitive ability of white clover for nutrients is probably related to differences in root morphology (Evans, 1977) and/or root CEC (Mouat and Walker, 1959). Researchers have also found that when K is present at high levels alfalfa dominates, while grass dominates when K is in limited supply (Hunt and Wagner, 1963; Macleod and Bradfield, 1963). Jones (1970) reasoned that the deep tap root system of alfalfa may give it the same advantage where nutrients such as P and S are leached below the depth from which shallow rooted companion crops can absorb them.

2.3.3. Water:

Competition for water usually occurs together with other forms of competition especially for light and nitrogen (Donald, 1963). Although grasses and clover explore approximately the top 140 cm of soil, the roots of clover are less ramified and hence the volume of soil explored is less than that of grasses (Evans, 1977). This may result in grasses having a competitive advantage over clover for water uptake.

Generally, temperate grasses are less affected by dry conditions than temperate legumes, with the exception of alfalfa (John's, 1972). Alfalfa competes well when grown with grasses where water is limiting (Chamblee, 1972; Smith and Steifel, 1977), as the long tap root of alfalfa enables it to obtain water from lower soil depths than grasses. Generally, grasses have longer, thinner, more finely branched roots than clovers, thus the former explore a greater volume of soil than clover for nutrients (particularly P, K, and S) and water uptake.

2.4. Techniques for injecting solutions into field crops:

Over the past decade several methods have been developed to inject solutions into plants. These attempts were initiated mainly so as to have control of the rate, time, and quantitiy of solutions being administered to the plants.

The first reported attempts to inject plants were from Grabau et al. (1986) working with soybean and Mackown and Van Sanford (1986) working with winter wheat. Grabau et al. (1986) used a pediatric intravenous kit to inject soybean plants with amino acid solutions. Syringe needles were used to inject the stems of soybean plants starting between nodes 3 and 4 and progressing upwards as the plant grew. The solution to be injected was suspended 1.5 m above the base of the plants. They succeeded in injecting an average of 51.2 mL per plant from the beginning of seed development until physiological maturity. Using the same technique as Grabau et al. (1986), Schon and Blevins (1987) were able to inject soybean plants with boron and calcium solutions at an average rate of 18.5 mL per week from the onset of flowering until complete senescence.

Boyle et al. (1991) developed a stem infusion technique for corn plants by which substantial amounts of water-soluble substances could be infused into the stems. The technique involved the addition of liquids through an infusion cavity made by removing approximately 10 mL of tissue and plugging in the hole with a rubber serum stopper. The infused solution was administered into the stem through a needle that penetrated the serum stopper. The liquids were gravity fed under pressure heads of up to 100 cm. Dilute solutions could be infused at a rate of up to 10 mL per infusion site.

In parallel work, Ma and Smith (1992) developed a method for adding nitrogenous solutions to barley plants using an infusion system in which the infusion occurs through the hollow peduncle internode. Plants were able to take up to 68 mL of the solution during a 20 day injection period. Using the same technique, Ma et al. (1994a) were able to perfuse substantial amounts of concentrated solutions into barley plants. Fourtan-pour and Smith (1995) extended this technique to full grown barley.

Ma et al. (1994b) developed a variation of the perfusion technique to perfuse field grown maize with concentrated sucrose solutions, which appeared to increase grain set for some hybrids. Recently, Zhou and Smith (1996) developed a new pressurized injection technique and injected corn plants with concentrated solutions of sucrose in water. The technique involves the injection of solutions through a hypodermic needle connected to a syringe. The pressure is supplied by ceramic bricks placed on the syringe plunger, which produces enough pressure to force the concentrated solution into corn stems. The average solution uptake was 5.1 mL d^{-1} per plant, and this was maintained for periods of up to 30 days.

2.5. Hypothesis:

The overall hypothesis of this research was that an improved understanding and manipulation of competitive relationships between and within plants will allow development of better cropping systems.

The following specific hypothesis were tested in this work:

1. Interseeding of forage legumes and grasses at early stages of corn development will reduce corn grain yield.
2. Different cover crops have different abilities to provide adequate cover under corn canopies.
3. Interseeded cover crops will suppress weed populations in grain corn
4. Cultivation in combination with cover crops would provides an efficient means of controlling weed populations.
5. Soybean plants can be injected with water soluble solutions through most of their growth and development.
6. Growing soybean plants under shade simulates the shading aspect of field conditions in an intercrop system and providing these shaded plants with sucrose and nitrogen, via stem injection, alleviates the detrimental effects of shading.
7. Injection of soybean plants with sucrose and nitrogen solutions throughout their reproductive period will reduce intra plant competition, resulting in a protracted reproductive period (delayed senescence) and greater yields.

2.6. Objectives:

The objectives of this research were:

- to determine when after corn seeding forage intercrops can be sown without causing corn grain yield reductions in a short season area,
- to compare the effects of interseeded forage species on corn grain yield,
- to screen 12 potential cover crops seeded at two dates for their potential efficacy in reducing weed populations in the absence of herbicide applications,
- to determine the combined effect of cultivation and cover crops in reducing weed populations,
- to test the possibility of injecting concentrated solutions of water soluble material into stems of soybean plants using a modified injection technique,
- to assess the effects of carbon supplementation, in the form of injected sucrose solutions and nitrogen supplementation on the growth and development of soybean plants,
- to determine the response of shaded soybean plants to increased levels of injected sucrose and nitrogen,
- to develop an understanding of competition for carbon and nitrogen within soybean plants,
- to determine the effects of injected sucrose and nitrogen on the senescence of soybean plants.

Preface to Chapter 3

This section is part of a manuscript by Abdin et al. (1996) submitted to the Agronomy Journal. The format has been changed to conform to a consistent format within this thesis. All literature cited is listed at the end of the thesis.

The potential use of forage legumes and grasses in grain corn in eastern Canada is being assessed. In this section the effects of growing twelve forages in combination with grain corn in a polyculture system and its effects on corn grain yield was investigated.

Chapter 3

YIELD AND YIELD COMPONENTS OF CORN INTERSEEDED WITH FORAGE LEGUMES AND GRASSES

3.0. Abstract :

Increasing concerns about the rising costs of pesticides, soil erosion and environmental pollution associated with corn production have led to the study of alternative production methods. Growing cover crops simultaneously with corn would address all of these concerns. Field experiments were conducted in 1993 and 1994 at two sites in Québec to determine the effects of interseeding twelve different forage legume and grass species or species mixtures on corn (*Zea mays* L.) yields and yield components. Fall rye (*Secale cereal* L.), hairy vetch (*Vicia villosa* Roth), a mixture of red clover (*Trifolium pratense* L.) and ryegrass (*Lolium multiflorum* Lam), a mixture of white clover (*Trifolium repens* L.) and ryegrass, subterranean *clover (Trifolium subterraneum* L.), yellow sweet clover (*Melilotus officinalis* Lam.), black medic (*Medicago lupulina* L.), Persian clover (*Trifolium resupinatum* L.), strawberry clover (*Trifolium fragiferum* L.), crimson clover (*Trifolium incarnatum* L.), alfalfa (*Medicago sativa* L.), and berseem clover (*Trifolium alexandrinum* L.) were seeded at two planting dates, 10 and 20 days after corn emergence. The experimental design was a split-plot randomized complete block with 4 replications at each site. The whole plots were the planting dates, the subplots were the twelve forage species, or species mixtures, and three control treatments; hand weeding, chemical weeding, and on-weeding. In 1993, at the Macdonald site, corn grain yields from

interseeded plots, except for crimson clover, were not different from the weeded controls. Crimson clover established well but competed strongly with the corn resulting in a yield reduction. In 1994, precipitation was relatively high and no yield differences were observed between any of the treatments in that year, presumably due to the decreased competition for moisture and, possibly, the effects of the legumes from the previous year. Yields at the l'Assomption site were consistently lower than those at the Macdonald site, due mainly to high weed densities at the l'Assomption site where interseeded forages were not able to effectively suppress weed populations. The number of grains per cob were not affected by most treatments, suggesting that yield differences were due to effects on either the synthesis or translocation of carbohydrates, leading to lower weight per seed values. Chlorophyll fluorescence measurements (F_v/F_m) indicated that corn plants were stressed when interseeded with crimson clover. There was no evidence of N transfer from the interseeded forage legumes to the associated corn.

3.1. Introduction :

Winter annual legumes and non legumes have been used as green manure and cover crops for centuries (Semple, 1928). Pieters (1927), reported that cover crops were used for soil and water conservation in China 2000 years ago. The practice of growing legume cover crops and including them in crop rotations has decreased due to the relatively recent emergence of chemical fertilizers and herbicides (Doran and Smith, 1991). The potential use of cover crops in modern corn production has received little research attention since the mid 1960`s. Increased concerns about rising costs associated with fossil fuels and pesticides, and developnig problems with soil erosion and environmental pollution have prompted researchers to look for alternate means of crop production which maintain yields while reducing costs and environmental damage. Cover crops have the potential to address all of theses concerns.

The inclusion of legume cover crops in rotations with cereals offers several benefits such as reduced soil erosion (Holderbaum et al., 1990; Wall et al., 1991), nitrogen contribution to the succeeding crop (Neely et al.; 1987,Decker et al.; 1994), improved soil organic matter and physical properties (Gosdin et al.; 1949, Reid and Goss,1981), and decreased preference of pest organisms for the main crop (Lambert et al., 1987).

The possible benefits of the cover crop species could be achieved by rotation with the main crop or by growing the two crops together on the same land during the same growing season. Although incorporating a legume into the rotation provides the benefits listed above, their economic value decreases when they are used primarily as an N source (Allison and Ott, 1987), since during the year they are grown, no salable crop is produced on that land, reducing the income of the producer (Hesterman et al., 1992). Interseeding forage legumes in grain corn such that the corn and forage crops are grown simultaneously is a possible alternative to crop rotation and may avoid some of the above problems while maintaining the useful benefits of cover crops. However interseeded legumes in corn can decrease both grain and silage yields. Schaller and Larson (1955) reported that early forage seeding in corn caused a reduction in corn yields. Nordquist and Wicks (1974) interseeded alfalfa simultaneously with silage corn and measured 20 to 50% reductions in corn yield due to competition from the forage crop. Scott et al. (1987), delayed forage seeding until the corn was 15 to 30 cm in height and reported no grain yield reduction. Claude et al. (1993) reported a corn yield reduction when red clover was interseeded with corn, due primarily to the inability of red clover to compete with the existing weeds. Although it is possible to grow winter annual legumes in temperate regions of the world, their use as cover crops is restricted by factors such as temperature and water availability (Smith et al., 1987) as well as competition with the crop under which they are grown. Legumes differ in their ability to establish well in an interseeding situation. Exner and Cruse (1993) reported that, when interseeded under corn, alfalfa and sweet clover usually established better and produced more cover than either red or alsike clover.

In legume grass mixtures, evidence of N transfer from the legume to the associated grass has been reported since the 1930's (Virtanen, 1933; Nicol 1934) through direct excretion of nitrogen from the legume roots (Wilson, 1941) or through the sloughing and decay of legume nodules (Butler and Bathrust, 1956). Patra et al. (1986) reported that 28% of the total N uptake of maize was obtained by transfer of fixed N by cowpea grown in association with maize. Using the ^{15}N dilution method, Martin et al., (1991) showed evidence of nitrogen transfer from nodulating soybean to corn and to non-nodulating soybean. Nitrogen transfer from interseeded forage legumes to associated corn plants could help to offset some of the potential negative effects of competition from other resources by the interseeded species.

The objective of the work reported here was to determine (i) when after corn seeding forage intercrops can be safely sown in a short season corn production area, and (ii) the effect of these interseeds on corn grain yield.

3.2. Materials and Methods:

An experiment evaluating the effects of interseeding forage crop species as ground cover on corn grain yield was conducted in 1993 and 1994 at two Québec, Canada, sites, one at the E. A. Lods Agronomy Research Center, McGill University, Macdonald Campus, and the other on the Agriculture and Agri-Foods Canada Research Farm at l'Assomption. The two sites are approximately 80 km apart. The soil types at the Macdonald site was a mixture of Chateauguay clay (fine loamy, mixed, nonacid, frigid, Hapludalf) and St. Bernard clay (fine loamy, mixed, nonacid, frigid, Eutrochrept). At l'Assomption, the soil type was a Soulange silt loam (fine-silty, mixed, nonacid, frigid Humaquept). Soil tests prior to planting showed that the soil at the Macdonald campus site had a pH of 5.5, and five t ha^{-1} of agricultural limestone was applied to raise the pH. Fertilizer was broadcast immediately prior to planting to achieve the recommended rates of 180, 37 and 100 kg ha^{-1} of N, P, and K, respectively. An additional two hundred and ten, and 95 kg ha^{-1} of 19-8-15 (N-P-K) were added to the soil through the corn seeder at the Macdonald and l'Assomption sites, respectively. The soil was harrowed 7 days before planting after which the lime and fertilizer were broadcast and disked in, to produce a smooth seed bed. The

Macdonald site was fallow the year before starting the experiment, while the l'Assomption site was pasture in 1992.

The experiment was conducted in a split-plot arrangement with 4 replications at each site. The two factors under study were the time of forage planting and the type of forage species. The whole plots comprised the two ground cover planting dates: 10 and 20 days after corn emergence. Scott and Burt (1985) suggested that interseeded forage species be sown when corn is 15 to 43 cm high, corresponding approximately to 20 days after seeding. However, in northern locations this leaves a shorter period for development and earlier seeding might be desireable. Thus, we also tested a 10 day after corn seeding date, when the corn was approximately 11 cm tall. The subplots were twelve forage species (Table 3.1) and three controls. The seeding dates for each site-year for each forage species at all site years are given in Table 3.2. Legumes were inoculated prior to planting with the appropriate commercial inoculant. In 1993, the three controls were hand weeded, chemically weeded and unweeded treatments. In 1994, a control consisting of mechanical weeding with a Rabe Werk cultivator (Rabe Werk Machinerie Agricole, St.-Césaire, QC, Canada) was added for each planting date. This control replaced the plots in which black medic had been seeded the previous year. The corn hybrid Pioneer 3921 was planted at a rate of 80,000 plants
ha^{-1}. The main plot size was 15m by 21 m and the subplot size was 3m by 7 m. Each sub plot consisted of 4 corn rows planted 75 cm apart with 16.4 cm between plants in the same row.

Corn was planted on May 13 and 11 in 1993 and on May 11 and 14 in 1994 at the Macdonald and l'Assomption sites, respectively. The corn was seeded with, a John Deere planter (model Max Emerge2 7200) at l'Assomption, and a Gaspardo planter (SP 510, Pordenone, Italy) at Macdonald. They were both adjusted to give a planting density of 80,000 seeds ha^{-1}. The 1994 experiment was planted at the same site as the 1993 one, and the plots of each treatment were planted in the same location each year. Cultivation was conducted weekly until forage seeding, with the Rabewerk cultivator (a rigid tine cultivator with a goose foot attachment). A mixture of metolachlor [2-chloro-N-(2-ethyl-6-methylephenyl)-N-(2-methoxy-1-methylethyl) acetamide] and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] were applied preemergence at a rate of 1.9 and 1.0 L ha^{-1}, respectively, to the chemically treated control plots using a bicycle-wheel plot sprayer. Average monthly rainfall and temperatures for each site year along with 30 year averages are given in Table 3.1. The forages were hand broadcast over the plots at their respective densities (Table 3.2).

Corn was harvested at maturity, during the second week of October in both years. Ears were harvested by hand from the two center rows of each plot leaving one meter unsampled at each end. Corn ears were shelled on site with a small plot combine (Wintersteiger America Inc., Lincoln, NE). Corn grain was then weighed to determine wet grain yield after which it was dried at 70°C to constant weight to determine the moisture content. Grain yield is reported on the basis of 0% moisture. A subsample of grain from each plot was ground to pass through a 2 mm mesh with a Wiley mill (A. H. Thomas Co., Philadelphia, PA.). These samples were used to determine the grain protein concentration (GPC) (g kg^{-1}) using Kjeldahl analysis (Tecator, Kjeltec 1030 auto analyzer, Sweden) (N x 6.25). Corn height was measured from the soil surface to the tip of the tassel on 3 randomly selected plants from the 2 middle rows of the plot.

Chlorophyll fluorescence was measured using a Morgan CF-1000 chlorophyll fluorescence measurement system (Morgan Scientific Inc., Andover, MA.). Five measurements were taken from each plot. Five corn plants per plot were sampled. One cuvette per plant was placed on the corn ear leaf from the two central rows of the plot. Cuvettes were left for about 10 minutes so that the leaves were acclimatized to darkness. The optical probe was then inserted in the cuvette and a reading was taken and used to determine the Fo (non-variable fluorescence), the Fm (maximal fluorescence), the Fv (variable fluorescence), and the ratio of Fv:Fm which is a measure of the photochemical efficiency of PSII (Photosystem II). This gives an indication of the stress status of the corn plants. Readings from the five samples were averaged to calculate the corn chlorophyll fluorescence for that plot. Leaf area index (LAI) was measured using the LAI-2000 plant canopy analyzer (LI-COR Inc., Lincoln, Nebraska, USA), measurements were made at silking. Six below-canopy readings with a 45° field-of-view cap were made to improve the spatial average. One reading was made above the corn canopy and 6 readings were made along diagonal intersects between the 2 central corn rows and above the interseeded forages to determine the LAI; corn readings were made at even intervals across the corn row. Another above canopy reading was made followed by 6 readings below the interseeded forage canopy to determine the LAI of the interseeded forages and the corn. The LAI was calculated from the logarithmic average of the canopy noninterceptance (below canopy reading divided by the above canopy reading) for each interval measured along the diagonal transect. The two transects on each side were then averaged to obtain an LAI value for the sampled plot.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1985) was used for analysis of variance of all data. Probabilities equal to or less than 0.05 were considered significant for main effects and interactions. The least significant difference (LSD) test was used to separate differences between treatment means if analysis of variance indicated the presence of such differences (Steel and Torrie, 1980).

3.3. Results and Discussion

There was substantially more precipitation in 1994 than in 1993 at both sites, and in 1993 precepitation at l'Assomption was slightly higher than at Macdonald. The average temperature was about the same for both years at that site (Table 3.2).

3.3.1. Grain yield:

In 1993 and 1994, at both sites, corn grain yields were not different between the two interseeding dates suggesting that the time of forage seeding, as long as it was reasonably delayed from the corn seeding date, did not effect grain yield. There was no seeding date by forage species interaction. Scott and Burt (1985) recommended that species such as alfalfa, hairy vetch and red clover be seeded when corn is 15 to 43 cm high. The weed densities observed under our experimental conditions (96-899 gm m^{-2}) suggests that earlier seeding of forages is possible, thereby allowing better ground cover and weed control. Overall, site years biomass production by the interseeded forage species was higher for early (620 kg ha^{-1}) than late (552 kg ha^{-1}) seeding.

In 1993 at the Macdonald Campus site, corn yields in the weedy control treatments were consistently lower than those of any of the other treatment (Table 3.3). At l'Assomption, the weedy control treatment had lower yields than all treatments except corn interseeded with subterranean, Persian, strawberry, and crimson clover. The l'Assomption site was a very weedy site (538 gm of dry weed biomass m^{-2} at l'Assomption versus 189 gm m^{-2} at Macdonald), and the former three clovers were unable to compete effectively with the emerging weeds. As a result, these clovers allowed substantial weed establishment (487 gm per m^{-2}), leading to reduced corn yields. Crimson clover was able to establish well despite severe competition from weeds. At l'Assomption, the rapid establishment of both weeds and crimson clover caused severe competition with corn, resulting in a yield reduction for this treatment. In 1993, at the Macdonald Campus site, except for the plots in which crimson clover was seeded, corn grain yields from the interseeded plots were not different from those of the hand weeded or chemically weeded controls. This is in agreement with Scott and Burt (1985) who reported no effect on corn yield due to seeding intercrops of red clover, alfalfa, yellow sweet clover, alsike clover, bird's foot trefoil, and hairy vetch. Exner and Cruse (1993) reported that corn yields were only reduced if the planting date for the forage legumes was early. Blevins et al. (1990) found that corn yields from plots interseeded in the fall with hairy vetch were actually higher than those of control plots. Guldan et. al. (1996) reported no reduction in the sweet corn yield, when interseeded with legume cover crops. In 1993, at l'Assomption, the weeded controls had consistently higher yields than any of the interseeded plots. Being a very weedy site, the reduction in corn yield in the interseeded plots probably resulted from competition from both the intereseeded forages plus the existing weeds.

In 1994, at the Macdonald site (Table 3.5), corn yields were not different among treatments, although the weedy control had the numerically lowest yield value (7 Mg ha^{-1}). There were no differences between the cultivated control and any of the hand, chemical or weedy controls. The favorable 1994 weather conditions probably minimized plant competition for water. Morris and Garrity (1993) reported that competition for water is one of three main factors in competition between components of intercrops. Zhou et al., (personal communication) reported that, in an intercrop system of corn and annual ryegrass, corn yields were not reduced when sufficient N and soil water were present. Exner and Cruse (1993) reported that soil moisture was more depleted under interseeded treatments than in monoculture corn checks when moisture was limiting and that in seasons of normal precipitation this depletion was not observed. In 1994, at Macdonald, mean yields were also higher than in 1993; the increase in yield was greater for interseeded treatments than the weeded controls indicating that some of the increase might be due to the contribution of the forages seeded in 1993 to soil nutrients and organic matter. Average increase in grain yield in previously interseeded plots was 9.2% higher in 1994 than 1993, compared with an 8.7% increase in grain yield of control treatments, suggesting that the more favorable weather conditions in 1994 were the cause of most of the 1994 increase, and that the residue from the interseeds was responsible for only 0.5% . However, this may be noteworthy after only one year. This is in agreement with the findings of Olson et al. (1986) who reported an increase in corn yields following a green manure of alfalfa and rye. Decker et al. (1994) have reported an increase in corn grain yield following cover crop legumes as compared with no cover crop. When corn was planted in a living mulch of crimson clover, Kumwenda et al. (1993) measured higher corn grain yields in1989 than 1988, with the increase being attributed mainly to a greater rainfall for 1989.

3.3.2. Corn height and leaf area:

In 1993, at Macdonald, the shortest corn plants were from the weedy control plots, which was consistent with the yield data. The reduced corn height was probably due to severe competition with weeds for water and nutrients. If a particular crop component in an intercrop system develops better access to limiting resources, there will be a tendency for that component to compete more strongly with and deny more resources to the other component (Donald, 1958; Milthorpe, 1961), causing a decrease in development, including height, and yield of the other component. Fukai and Trenbath (1993) explained that shading of one component by another would lead to reduced root development for the shaded crop leading to further reduced height. Hand weeded control plots produced the tallest corn plants, although they were not different from plots interseeded with subterranean clover, yellow sweet clover, Persian clover and alfalfa, indicating that these three clovers did not compete vigorously with corn. Corn plant heights in 1994 were not affected by the seeded forages, except for plots in which fall rye was interseeded; probably the soil moisture availability that year minimized the cover crop effect on corn plant heights (Table 3.5). Corn heights in the cultivated control plots were not different from the other control treatments. Midmore (1993) suggested that in the presence of adequate soil moisture, agronomic modifications could be maximized so as to mitigate competition for light. Corn plants in Persian clover plots were taller than those from the mechanically cultivated control plots and plots in which fall rye was seeded. Leaf area indices at the Macdonald site were higher for all treatments than those of the l'Assomption site indicating better corn establishment and growth at the former site (Table 3.6). At the Macdonald site, plots interseeded with alfalfa had higher leaf area indices than any of the other interseeded treatments and they were comparable to those from the hand and chemically weeded controls. At l'Assomption, there were no differences in corn leaf area index among the interseeded treatments. Average corn leaf area index from strawberry clover and alfalfa treatments were higher than those of the weedy control.

3.3.3. Yield components :

In 1993 and 1994, at Macdonald, the highest average number of ears per plant was recorded for the hairy vetch interseeded plots which was different from all other treatments except those of the cultivated control, crimson clover, red clover-ryegrass mixture and the hand weeded control in 1994 and the white clover/ryegrass, strawberry clover, berseem clover, and the hand weeded control in 1993 (Tables 3.3 and 3.5). In 1994, there were no differences among the four control treatments. At l'Assomption, the hand weeded control plots had the highest average number of ears per plant, but it was only different from strawberry clover interseeded plots. The weedy control had the fewest ears.

In 1993, at Macdonald, the weedy control had the lowest 100 grain weight of all treatments. Berseem clover interseeded plots produced lower test weights than those of the hand weeded and chemically weeded control plots as well as from plots in which subterranean clover, yellow sweet clover, Persian clover, and crimson clover were seeded. Although numerically lower, the average grain yield of berseem clover interseeded plots was not statistically different from the former treatments.

At l'Assomption, the lowest test weights were obtained from the weedy control plots, followed by strawberry clover interseeded plots which had lower test weights than those of the hand weeded and chemically weeded control plots, as well as plots interseeded with a mixture of red clover and rye grass, and crimson clover.

These results suggest that under favorable conditions corn plants are able to circumvent the effects of lower cob numbers and lower test weight, caused by competition with the forages, by compensating with the production of bigger ears. However, under severe competition from weeds and forage plants, corn plants could not manage complete compensation and yields were reduced. The treatments had little effect on the number of grains per cob, suggesting that none of the treatments affected corn plants at the seed setting stage, and further suggesting that the effects occured at a later stage, during grain filling. In 1993, at the Macdonald site, crimson clover had a more pronounced effect on the cob grain yield than any of the other interseeds and its presence resulted in the lowest corn grain yields of any interseeded forage. In 1993, at the Macdonald site, the cob grain weight of the crimson clover treatments, was lower than the subterranean clover, black medic and Persian clover treatments, suggesting that crimson clover is very competitive with corn. Subterranean clover was least competitive and resulted in the highest cob yield and number of grains per cob. In 1994, at the Macdonald site, the highest average number of ears per plant was recorded from the hairy vetch interseeded plots. These values were higher than those from all other treatments except for the red clover-ryegrass mixture cultivated control, crimson clover, and the hand weeded control.

Hashemy and Herbert (1992) reported that shading had no effect on the row number per ear as well as the number of kernel rows per ear, and that the primary effect of shading was through a reduction in photosynthesis which reduced assimilate supply for yield development. Hatfield et al. (1965) stated that grain yield is a function of synthesis, translocation and conversion of photosynthate into the grain. Kiniry et al. (1990) reported an increase in weight per grain when the grain number was artificially reduced, however they suggested that a complex relationship existed between assimilate supply and weight per grain.

3.3.4. Chlorophyll Fluorescence :

In 1994, at the Macdonald sit,e chlorophyll fluorescence (F_v/F_m) of corn plants interseeded with crimson clover was lower than those from the chemically weeded control plots (Table

3.6). Although there was no significant reduction in grain yield, the chlorophyll fluorescence measurements indicated that the corn plants were more stressed when interseeded with crimson clover. This could be attributed to the greater competitive ability and the better establishment of the crimson clover in comparison to forages such as fall rye, strawberry clover, or alfalfa. The chlorophyll fluorescence values obtained also provide an indication of photosynthetic status of the corn plants. Ireland et al. (1989) showed a linear relationship between carbon dioxide assimilation and the variable fluorescence ratio (F_v/F_m). Similarly, Seaton and Walker (1990) derived a single curvilinear relationship between fluorescence and carbon assimilation that would allow the measurement of photosynthesis without resorting to the analysis of gaseous exchange between the leaf and the atmosphere. Therefore, the rates of corn photosynthesis of corn plants interseeeded with crimson clover may have been lower than those from the chemically weeded controls, indicating the stressful nature the corn was under when inteseeded with crimson clover.

3.3.5. Grain protein content:

In 1994 there were no differences among treatments for grain protein concentration, except for hairy vetch interseeded plots where the corn grain had a lower protein concentration than the mechanically cultivated control, subterranean clover and red clover/rye grass mixture plots. This lack of difference argues against maningful levels of nitrogen transfer from any of the forage legumes to associated corn plants.

3.3.6. Relative corn maturity:

Interseeding did not appear to affect the relative maturity of corn, measured as percentage grain moisture at harvest (Mather and Kannenberg 1989). In 1993 the weedy control treatment at Macdonald had higher grain moisture than any of the other treatments. In 1994 this effect was not observed, presumably due to the effects of higher rainfall that year.

3.3.7. Conclusions:

Under the conditions of this experiment, corn yields were not affected by the time of forage seeding but were affected by the type of forage species used as an intercrop. Interseeded forages were not able to compete effectively with weeds when planted in fields heavily infested with them and other methods of weed control should be conducted prior to use of interseed forages to control weeds in heavily infested fields. When there was competition for moisture, crimson clover was found to be too competitive with corn at the seeding rates used in this experiment. Under conditions of adequate moisture, corn yields were not affected by the vigorous growth of crimson clover, or any of the interseeded forages. There was no evidence of N transfer or residue effects on grain protein.

Table 3.1. Total monthly precepitation and average temperature recorded at Macdonald and l'Assomption during 1993 and 1994 and 30 year averages.

Year	May	June	July	August	September	Total	May	June	July	August	September
			Precepitation mm Macdonald						Temperature °C Macdonald		
1993	79.1	74.8	94.6	57.2	119.2	424.9	13.3	17.6	21.4	20.5	13.9
1994	148	194	61.3	99.9	105.5	607.8	12.1	18.9	21.3	18.0	14.3
Average *	70.6	88.3	89.7	92.6	97.9	439.1	13.1	18.1	21.1	19.8	14.7
			l'Assomption						l'Assomption		
1993	95.6	74.2	75.4	95.6	89.1	429.9	12.7	17.4	20.7	20.3	13.6
1994	93.8	285.9	122.8	67.8	121.6	691.9	11.8	19.3	21.0	19.2	14.7
Average *	72.5	87.0	84.5	94.4	84.6	423.0	12.3	17.5	20.2	18.8	13.8

*30-yr averages

Table 3.2. Seeding rates of the interseeded forage legumes and grasses in 1993 and 1994 at Macdonald and l'Assopmtion

Cover crop names			
Common Name	Cultivar	Latin Name	Seeding rate kg ha^{-1}
Fall rye	Prima	*Secale cereale* L.	110
Hairy vetch	Canada No. 1	*Vicia villosa* Roth.	30
Red clover + ryegrass	Khun + Marshall	*Trifolium pratense* L. + *Lolium multiflorum* L.	10 + 8
White clover + ryegrass	Ladino + Marshall	*Trifolium repens* L. + *Lolium multiflorum* L.	7 + 8
Subterranean clover	Northam	*Trifolium subterraneum* L.	12
Yellow sweet clover	Canada No. 1	*Melilotus officinale* L.	7
Black medic	Canada No. 1	*Medicago lupulina* L.	15
Persian clover	Canada No. 1	*Trifolium resupinatum* L.	10
Strawberry clover	Salina	*Trifolium fragiferum* L.	7
Crimson clover	Canada No.	*Trifolium incarnatum* L.	22
Alfalfa	Nitro	*Medicago sativa* L.	12
Berseem clover	Canada No. 1	*Trifolium alexandrinum* L.	20

Table 3.3. Influence of interseeded forage legumes and grasses on corn grain yield and yield components at Macdonald in 1993.

Forage Species	Grain Yield	Moisture	Cobs per Plant	Height	Cob grain weight	Grains per Cob	100 Grain weight
	Mg ha^{-1}	g kg^{-1}		cm	gm	grain cob^{-1}	gm
Fall rye	8.0 abc	261 bc	1.02 bc	249.3 e	99.5 abc	391.0 ab	25.5 ab
Hairy vetch	8.7 ab	254 bcd	1.13 a	258.7 bcde	91.7 bcd	354.8 ab	25.5 ab
Red clover + Ryegrass	8.3 ab	255 bcd	1.03 bc	256.3 bcde	100.5 abc	396.5 ab	25.2 ab
White clover + Ryegrass	8.4 ab	266 b	1.08 ab	258.6 bcde	97.8 abc	380.7 ab	25.6 ab
Subterrranean clover	8.9 a	255 bcd	1.01 bc	264.0 abc	110.9 a	424.8 a	26.1 a
Yellow sweet clover	7.7 bc	260 bcd	1.02 bc	266.7 ab	93.6 abcd	375.0 ab	26.0 a
Black medic	8.5 ab	248 bcd	1.03 bc	253.6 cde	103.1 ab	410.2 ab	25.2 ab
Persian clover	8.1 abc	253 bcd	0.97 cd	261.2 abcd	104.6 ab	401.7 ab	26.2a
strawberry clover	8.6 ab	259 bcd	1.06 abc	256.3 bcde	102.4 abc	402.8 ab	25.4 ab
Crimson clover	7.0 c	251 bcd	1.04 abc	252.2 de	84.48 cd	339.1 b	26.2 a
Alfalfa	7.9 abc	261 bcd	1.00 bc	262.1 abcd	100.4 abc	382.5 ab	25.9 ab
Berseem clover	7.8 abc	255 bcd	1.08 ab	259.0 bcde	91.7 bcd	368.2 ab	24.9 b
Hand weeded control	8.6 ab	243 cd	1.07 ab	271.7 a	101.0 abc	396.8 ab	26.0 a
Chemically weeded control	8.7 ab	242 d	1.04 bc	261.2 abcd	106.1 ab	405.7 ab	26.1 a
Weedy control	5.4 d	285 a	0.90 d	231.2 f	79.0 d	359.2 ab	22.2 c
LSD (0.05)	1.2	18	0.09	11.4	18.4	81.7	1.0

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$)

Table 3.4. Influence of interseeded forage legumes and grasses on corn grain yield and yield components at l'Assomption 1993.

Forage Species	Grain Yield	Moisture	Cobs per Plant	Height	Cobs grain weight	Grain per cob	100 Grain Weight
	$Mg\ ha^{-1}$	$g\ kg^{-1}$		cm	gm	$grain\ cob^{-1}$	gm
Fall rye	7.5 b	255 ab	1.05 ab	260.0 ab	90.4 abc	373.1 abc	24.2 bc
Hairy vetch	7.0 bc	280 a	1.0 ab	252.0 bc	87.5 bc	380.7 abc	23.4 bc
Red clover + Ryegrass	7.2 bc	245 bc	1.03 ab	262.0 ab	87.6 bc	356.6 abc	24.6 b
White clover + Ryegrass	7.5 b	259 ab	1.00 ab	249 bc	95.2 ab	398.8 a	23.8 bc
Subterrranean clover	6.3 cd	255 ab	1.04 ab	262.8 ab	76.9 c	323.5 c	23.7 bc
Yellow sweet clover	7.5 b	263 ab	1.01 ab	239.5 cd	93.6 abc	395.3 ab	23.6 bc
Black medic	7.1 bc	252 bc	1.06 ab	259.5 ab	85.1 bc	357.5 abc	23.8 bc
Persian clover	6.7 bcd	255 ab	1.01 ab	253.0 bc	83.0 bc	345.0 abc	24.1 bc
Strawberry clover	6.8 bcd	249 bc	0.98 b	253.4 bc	87.4 bc	383.5 abc	22.7 c
Crimson clover	6.4 cd	258 ab	1.00 ab	248.0 bc	82.0 bc	326.8 bc	24.9 ab
Alfalfa	6.9 bc	254 ab	0.99 ab	246.0 bc	86.7 bc	380.1 abc	22.7 c
Berseem clover	7.6 b	251 bc	1.02 ab	252.0 bc	93.5 abc	388.2 abc	24.1 bc
Hand weeded control	9.0 a	225 cd	1.09 a	273.0 a	106.5 a	406.8 a	26.5 a
Chemically weeded control	8.7 a	214 d	1.03 ab	261.1 ab	106.5 a	402.6 a	26.5 a
Weedy control	5.8 d	281 a	0.85 c	226.4 d	76.4 c	362.75 abc	20.7 d
LSD (0.05)	1.0	27	0.10	27.7	17.3	70.3	1.5

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$)

Table 3.5. Influence of interseeded forage legumes and grasses on corn grain yield and yield components at Macdonald in 1994.

Forage Species	Grain Yield	Moisture	Cobs per plant	Height	Cobs grain weight	Grain per cob	100 Grain weight	Grain Protein Content
	Mg ha^{-1}	g kg^{-1}		cm	gm		gm	g kg^{-1}
Fall rye	8.8 a	237 ab	1.03 b	255.8 c	107.1 ab	346 a	31.2 abcd	85 ab
Hairy vetch	9.0 a	223 abcd	1.14 a	271.9 abc	110.1 ab	335 a	31.9 ab	80 b
Red clover + Ryegrass	9.3 a	214 bcd	1.06 ab	275.1 ab	109.9 ab	360 a	30.8 abcd	92 a
White clover + Ryegrass	8.8 a	227 abcd	1.03 b	264.9 abc	105.3 ab	345 a	32.5 a	86 ab
Subterrranean clover	8.7 a	231 abc	0.99 b	271.9 abc	116.5 a	377 a	30.8 abcd	92 a
Yellow sweet clover	8.7 a	217 bcd	1.02 b	270.3 abc	114.5 a	382 a	31.3abc	83 ab
Cultivated control	9.4 a	202 cd	1.07 ab	262.4 bc	110.6 ab	353 a	31.9ab	93 a
Persian clover	8.6 a	252 a	0.99 b	280.5 a	109.5 ab	354 a	31.4 abc	87 ab
Strawberry clover	8.9 a	226 abcd	1.01 b	272.3 ab	109.1 ab	359 a	29.4 bcd	83 ab
Crimson clover	9.7 a	207 cd	1.07 ab	270.8 abc	114.0 a	352 a	31.4 abc	89 ab
Alfalfa	8.8 a	227 abcd	1.03 b	269.6 abc	107.4 ab	363 a	29.4 bcd	84 ab
Berseem clover	9.4 a	198 d	1.00 b	272.6 ab	113.5 a	379 a	28.8 d	91 ab
Hand weeded control	8.9 a	229 abc	1.05 ab	272.9 ab	108.5 ab	370 a	29.4 bcd	86 ab
Chemically weeded control	8.7 a	202 cd	1.01 b	278.0 ab	107.9 ab	360 a	30.9 abcd	89 ab
Weedy control	7.0 a	216 cde	0.99 b	267.6 abc	99.2 b	335 a	30.18 abcd	87 ab
LSD (0.05)	1.1	29.0	0.1	16.3	11.9	51.0	2.4	11

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$

Table 3.6. Effect of interseeding forage legumes and grasses on corn chlorophyll fluorescence and leaf area index (LAI) at Macdonald and l'Assomption in 1994.

Forage Species	Macdonald		l'Assomption	
	Chlorophyll Fluorescence	Leaf Area Index	Chlorophyll Fluorescence	Leaf Area Index
Fall Rye	0.8090 ab	3.39 bc	0.8366 a	2.45 ab
Strawberry clover	0.8222 ab	3.29 bc	0.8219 a	2.58 a
Crimson clover	0.7901 b	3.53 bc	0.8313 a	2.45 ab
Alfalfa	0.7911 ab	3.98 a	0.8257 a	2.67 a
Cultivated control	0.8379 ab	3.31 bc	0.8393 a	2.50 ab
Hand weeded control	0.8100 ab	3.70 ab	0.8440 a	2.59 a
Chemically weeded control	0.8428 a	4.09 a	0.8188 a	2.60 a
Weedy control	0.8141 ab	3.18 c	0.8314 a	2.03 b
$LSD_{0.05}$	0.0524	1.8	0.0329	0.5

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Preface to Chapter 4

This section is part of a manuscript by Abdin et al. (1997) submitted to the journal Weed Technology. The format has been changed to conform to a consistent format within this thesis. All literature cited is listed at the end of the thesis. Each table or figure for chapter 4 is presented at the end of this chapter.

After assessing the feasibility of growing forage legumes and grasses with grain corn and its effect on grain yield in chapter 3, this chapter examines the potential use of some of the interseeded forages as weed suppressers in grain corn. The same design and treatments used in chapter 3 were used in this chapter.

Chapter 4

COVER CROPS AND INTERROW TILLAGE FOR WEED CONTROL IN SHORT SEASON CORN

4.0. Abstract:

Weed competition can cause substantial reductions in corn yields. Rising herbicide costs and increasing concerns regarding herbicide pollution of ground water have prompted the search for alternative means of weed control that are effective, environmentally safe, and economically feasible. Interseeding corn with cover crops or a combination of interrow tillage and interseeded cover crops are possible alternative methods of weed control. Field experiments were conducted in 1993 and 1994 at two sites in Québec to determine the effects of planting twelve forage legumes and grasses with corn (*Zea mays* L.) on weed control. Fall rye (*Secale cereale* L.), hairy vetch (*Vicia villosa* Roth), a mixture of red clover (*Trifolium pratense* L.) and ryegrass (*Lolium multiflorum* Lam), a mixture of white clover (*Trifolium repens* L.) and ryegrass, subterranean clover (*Trifolium subterraneam* L.), yellow sweet clover (*Melilotus officinalis* Lam.), black medic (*Medicago lupulina* L.), Persian clover (*Trifolium resupinatum* L.), strawberry clover (*Trifolium fragiferum* L.), crimson clover (*Trifolium incarnatum* L.), alfalfa (*Medicago sativa* L.), and berseem clover (*Trifolium alexandrinum* L.) were seeded at two planting dates, 10 and 20 days after corn emergence. Interrow cultivation was carried out weekly until forage seeding, with a final

cultivation being conducted just prior to forage seeding. The study examined the potential of interrow tillage plus cover crops to reduce weed populations in maize without reducing the grain yield. The experimental design was a split-plot randomized complete block with 4 replications at each site. The whole plots were planting dates, and the subplots were the twelve forage species and three control treatments [hand weeded, chemically weeded (atrazine plus dual), and non weeded]. Cover crop planting date did not affect corn yields or the ability of interrow tillage plus cover crops to suppress the development of weed populations. Except for 1993 at l'Assomption, interrow tillage plus cover crop treatments had consistently lower weed biomasses than the non-weeded control. Most of the weed control was due to the inter row cultivation performed prior to seeding of the cover crops. However, the cover crops did provide some additional weed control and should have provided other soil benefits as well. The lowest weed populations occured in the herbicide treatment plots. Crimson clover was a promising cover crop as it was able to compete effectively with weeds, however, in 1993 crimson clover reduced corn yields. On the other hand, hairy vetch did not provide adequate cover or reduce weed populations. Hand weeded and chemically weeded treatments resulted in higher corn yields than any of the cover crop treatments and the weedy control treatment at the more weedy of the two sites.

4.4. Introduction:

Weeds represent an important variable in corn production, both economically and ecologically. Weed competition can cause yield reductions of up to 70% in corn grain yields (Teasdale, 1995). During the first half of this century the most common methods of weed control were rotary hoeing and interrow cultivation. The effectiveness of interrow cultivation in suppressing weed populations in corn is well documented (Wilson, 1993). However, cultivation represents an additional cost for the producer due to the consumption of fossil fuels (Lybecker et al., 1988). Frequent interrow cultivation is also associated with increased soil erosion as soil particles are more susceptible to dispersion after tillage (Dabney et al., 1993; Fuller et al., 1995). Soil disturbance can also increase weed germination (Roberts and Potter, 1980).

As herbicides became available, they gradually replaced cultivation as a method to control undesirable vegetation (Sprague, 1986). This was primarily due to their efficiency and convenience. However, ground and surface water pollution by pesticides are cause for concern (Hallberg, 1989), and corn herbicides have been among the pesticides most frequently detected in these waters (National Research Council, 1989) . Improving water quality and decreasing herbicide carryover is one of the most imporatnt environmental issues for farmers and agriculture researchers (Stoller et al., 1993).

Environmental concerns and the increasing costs of pesticides and fossil fuels have prompted growers and researchers to investigate alternative means for the development of environmental, economical, and effective weed management systems (Riggleman, 1987). Clements et al. (1995) found that most alternative weed control measures are more energy efficient than convetional practices, and that elimination of herbicide use could conserve energy while sustainig acceptable yields.

An alternative to herbicides and conventional cultivation is the use of cover crops between the crop rows. De Haan et al. (1994) suggested that spring seeded smother crops can reduce weed populations by up to 80% with little effect on corn yield. In addition to providing adequate cover to reduce soil erosion (Wall et al., 1991), legume cover crops improve soil nutrient status through addition of organic nitrogen (Holderbaum et al., 1990; Brown et al., 1993) via fixed atmospheric nitrogen which improves soil physical properties (McVay et al., 1989; Latif et al., 1992). Incorporating legume cover crops also increases the yield of the succeeding crop (Decker et al., 1994; Bollero and Bullock, 1994). In addition, cover crops can suppress weed populations by competing for light (Teasdale, 1993) water, and nutrients (Mayer and Hartwig, 1986) and through the production of allelopathic compounds (White et al., 1989) .

Studies in the early 1980's indicated that certain grass and legume species were well suited as cover crops for corn (Mt. Pleasant, 1982). Scott et al. (1987) reported that interseeding annual rye grass (*Lolium multiflorum* Lam.), medium red clover (*Trifolium pratense* L.), or a combination of the two provided good ground cover and dry matter production without affecting the corn grain yield if they were seeded when the corn was 15 to 30 cm high. Hoffman et al. (1993) reported that a hairy vetch (*Vicia villosa* Roth.) cover crop reduced weed biomass by 96% without causing any reduction in the corn yield when the vetch was planted in May or June. Teasdale (1993) reported that hairy vetch residues inhibited the establishment of common lamb's quarter (*Chenopodium album* L.) without affecting corn establishment. Other legumes, such as subterranean clover, have been reported to provide equal or better weed control in no till corn than herbicide treatments without a decrease in corn yields (Enache and Ilnicki, 1990; Ilnicki and Enache, 1992). Johnson et al., (1993) reported that a hairy vetch and rye cover provided good weed control in no till corn production. Palada et al. (1982) reported a 75% decrease in the number of weeds present when corn was interseeded with red clover or hairy vetch. Rye has good potential for suppressing weeds due to its allelopathic activity. Samson (1991) reported that interseeding ryegrass with corn consistently reduced weed biomass by about 50%. A recent study by Mohler and Callaway (1995) reported that the presence of fall rye (*Secale cereale* L.) decreased the seed production of *Portulaca oleraceae* L.in one year.

Although cover crops decrease weed populations, they usually require suppression the following season in order to minimize competition with the following crop, as in many cases cover crops, if left untreated for the next season, will compete with and reduce the yield of the following crop (Eberlein et al., 1992 ; Teasdale, 1993). Cover crop suppression is usually provided through the addition of herbicides (White and Worsham, 1990) as mowing may not be effective in controlling the growth of some cover crops (Hoffman et al., 1993). Recent research has concentrated on the method of suppression and the types and doses of herbicides to be used for the cover crop suppression, but this has raised a new set of environmental concerns.

Interrow cultivation can provide adequate weed control in corn. Forcella et. al. (1992) reported that interrow cultivation controlled up to 82% of the weeds in wide row crops such as corn. Greater than 85% weed control was reported by Parks et. al. (1995) when cultivation was combined with lower herbicide application rate. In an effort to reduce herbicide use, our study focuses on eliminating the use of herbicides and alternatively utilizing cover crops in combination with interrow tillage as an effective means of weed control.

Therefore, the objective of this experiment was to screen 12 cover crops seeded at two dates for their potential efficacy in reducing weed populations in the absence of herbicide application, and their effects on corn grain yields.

4.2. Materials and Methods:

An experiment evaluating the effects of interseeding forage crop species as ground cover, in conjunction with interrow tillage, for weed control in corn was conducted in 1993 and 1994 at two Québec (Canada) sites, the E. A. Lods Agronomy Research Center, McGill University, Macdonald Campus, and on the Agriculture and Agri-Foods Canada Research Station at l'Assomption. The two sites are approximately 80 km apart. The soil type at the Macdonald site was a mixture of Chateauguay clay (fine loamy, mixed, nonacid, frigid, Hapludalf) and St. Bernard clay (fine loamy, mixed, nonacid, frigid, Eutrochrept). At l'Assomption, the soil type was a Soulange silt loam -(fine-silty, mixed, nonacid, frigid Humaquept). Soil tests prior to planting showed that the soil at the Macdonald campus site had a pH of 5.5. Five t ha^{-1} of agricultural limestone was applied to raise the pH. Two hundred and ten, and 95 kg ha^{-1} of 19-8-15 (N-P-K) were added to the soil through the corn seeder at the Macdonald and l'Assomption sites, respectively. Additional fertilizer was broadcast immediately prior to planting to achieve the recommended rates of 180, 37 and 100 kg ha^{-1} of N, P, and K, respectively. The soil was harrowed 7 days before planting after which the lime and fertilizer were broadcast and disked in to produce a smooth seedbed. The Macdonald site was fallow the year before starting the experiment, and the l'Assomption site was seeded with pasture the previous year.

The experiment was conducted in a split-plot randomized complete block design with 4 replications at each site. The two factors under study were the time of forage planting and the type of forage species. The whole plots comprised the two ground cover planting dates: 10 and 20 days after corn emergence. Scott and Burt (1985) suggested that interseeded forage species be seeded when corn is 15 to 43 cm high, corresponding approximately to the 20 day seeding. However, in northern locations this leaves a shorter period for cover crop development and earlier seeding would be desireable. Thus, we have also tested a 10 day after corn emergence. At this stage, the corn was approximately 11 cm tall. The subplots were twelve forage species and three controls (Table 4.2). The seeding dates for each site-year and the seeding rates used at all four site years are given in table 4.2. Legumes were inoculated prior to planting with the appropriate commercial inoculant. In 1993 the three controls were a hand weeded, chemically weeded and unweeded treatment. In 1994, a control consisting of mechanical weeding with a Rabe Werk cultivator (Rabe Werk Machinerie Agricole, St.-Césaire, QC,Canada) was added for each planting date. This control was seeded on plots on which black medic had been seeded in 1993. The corn hybrid Pioneer 3921 was planted at a rate of 80,000 plants ha^{-1}.

The main plot size was 15 m by 21 m and the subplot size was 3 m by 7 m. Each sub plot consisted of 4 corn rows planted 75 cm apart with 16.4 cm between plants.

Corn was planted on May 11 and 13 in 1993 at the l'Assomption and Macdonald sites, respectively. In 1994, corn was planted on May 11 and 14 at Macdonald and l'Assomption, respectively. At the Macdonald site, a corn seeder (Gaspardo SP 510, Pordenone, Italy) was used and was adjusted to give a planting density of 80,000 seeds ha^{-1}. At the l'Assomption site, a John Deere planter (model Max Emerge2 7200) was used and was adjusted to the same seeding rate as that used at Macdonald. The 1994 experiments were planted on the same sites used in 1993 ones treatment plots were planted in the same location each year. Cultivation was conducted weekly until forage seeding, with the Rabewerk cultivator. A mixture of Dual Metolachlor [2-chloro-6'-ethyl-N-(2-methoxy-1-methylethyl) aceto-*O*-toluidide] and atrazine [6-chloro-N-ethyle-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] were applied preemergence at a rate of 1.9 and 1.0 L ha^{-1}, respectively, to the chemically treated control plot using a bicycle-wheel plot sprayer. Forages were hand broadcast over the plots at their respective densities (Table 4.1). Crop growth was dependant on precipitation; no irrigation water was applied. Total monthly rainfalls and average temperatures for each site year along with 30 year averages are given in table 4.1.

Corn was harvested at harvest maturity, during the second week of October in both years. Two quadrats of 0.5 m by 0.2 m were placed in each plot, one between and parallel to the corn rows, and the other on one of the two middle rows. During the second week of August, a destructive sample was taken from the quadrats and the weeds and forages were counted, identified and grouped as either dicots or monocots. Each of these groups was counted separately and placed in paper bags for drying. This procedure was used for weeds collected from both the area between and on the corn rows. The harvested samples were dried to a constant weight at 70°C for 2 days in a forced air dryer, after which they were weighed and their biomass recorded.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1985) was used to conduct an analysis of variance for all data reported. Probabilities equal to or less than 0.05 were considered significant for main effects and interactions. The Waller-Duncan test of significance was used to separate differences between treatment means if analysis of variance indicated the presence of such differences (Steel and Torrie, 1980).

4.3. Results and Discussion:

4.3.1. Climate:

The weather was wetter during 1994 than 1993 (table 2), and the l'Assomption site had more precepitation during the growing season than the Macdonald site in both years. The average temperature was about the same for both years.

4.3.2. Sites:

The weed populations at Macdonald were lower than those at l'Assomption and could be reduced successfully by cultivation. The main dicotyledonous were common lambsquarter (*Chenopodium album* L.) and common cocklebur (*Amaranthus retroflexus*

L.), and the main monocotyledonus weeds were *Setaria glauca* L., *Setaria viridis* L., and *Panicum cappilair* L. The l'Assomption site was heavily infested with weeds; quack grass (*Elytrigia repens* L.), common lambsquarter (*Cheneopdium album* L.) and foxtails (*Setaria* spp.) were the prodiminant weeds. These were not successfully controled by initial cultivation and had a profound effect on the efficacy of the tillage cover crop system.

4.3.3. Weed population and biomass:

Weed biomasses were lower in 1994 than 1993. This was probably due to the extra cultivation the plots received immediately prior to seeding the cover crops in 1994, which is in agreement with results obtained by Pava and Ulanday, (1993) . In 1993 at both locations mean weed biomass and population was higher on the corn row than between the rows (Tables 4.3, 4.5,and 4). This was particularly evident in 1993, and could be attributed to the better growing season and weather conditions in 1994 than 1993. However, examination of the weed components showed that this was not always true for all weed types. At Macdonald, there was a greater biomass of monocotolydonous weeds between the rows than on the rows. The reason for the higher weed biomasses on rows than between rows was probably due to interrow cultivations. In 1993, the cultivator blades that were passed between the corn rows were held in an upward position so as to minimize corn damage. As a result, the rows themselves and the space near the rows were not subject to cultivation related weed control, allowing weeds to establish and grow better there. In 1994, the blades were put in a downward position so as to cultivate the area near the corn rows more vigorously. This lead to a lower overall weed biomass on the rows compared with 1993 (tables 4.4 and 4.2). Mulder and Doll (1994) reported that row crop cultivators controlled between row weeds more effectively than on-row weeds.

Weed control in plots receiving the herbicide treatment was higher than any of the other treatments. Ninety five and ninety one percent of the weeds were controled by the herbicides in 1993 and 1994, respectively. In 1993 and 1994 at the Macdonald site, the combination of covercrops and cultivation controled 77 and 80 % of the weeds, respectively. At the l'Assomption site, cover crops plus tillage provided 76% weed control in 1994. Ilnicki and Enache (1992) reported that subterranean clover, which was able to control approximately 80% of the fall panicum and Ivy leaf morning glory in minimum tilled corn. Due to the relatively high weed infestation in 1993 at l'Assomption the combination of interrow tillage and cover crops were only able to control only 30 % of the weeds.

Inclusion of the interrow tillage control treatment in 1994 allowed us to separate the tillage and cover crop effects. In 1994, the combination of cover cover crops and interrow tillage controled 80 and 75% of the weeds at the Macdonald and l'Assomption sites respectively compared with the weedy control. Cultivation alone controled 70 and 80% of the weeds present at the Macdonald and l'Assomption sites, respectively. Thus, the cover crops alone were responsible for only about 10% of weed control at Macdonald. However, this was not true for the highly infested site as l'Assomption, as the interrow cultivation alone was able to control more weeds than interrow cultivation in combination with forage crops, except for treatments including crimson clover, alfalfa, or white clover plus rye grass. Indicating an effectiveness for using cover crops in fields with low weed populations but not for weedy ones.

Dicotolydonous weed biomasses were generally higher than those of monocotolydonous weeds except at l'Assomption in 1994. In 1993 at both locations weed population followed the same pattern as weed biomass, being higher on the corn rows than between the rows. In 1994 at l'Asssomption, the weed populations on the corn rows were less than those between the rows (Table 4.6), although the on row weed biomass was higher. This indicated that the on row population was composed of fewer larger plants than the between row population.

In both years at the Macdonald site and in 1994 at the l'Assomption site, all forage plus cultivation treatments were able to suppress weed population and biomass to levels that were lower than the non-weeded control. In 1993, at l'Assomption, the interseeded cover crops were not able to compete effectively with the weeds due to the severe weed infestation at that site. Chemical treatment was the only effective weed control method at the l'Assomption site (Table 4). In both years at the Macdonald site, plots interseeded with crimson clover had lower weed populations than any of the other interseeded forages. In spite of the treatment at l'Assomption, the weed infestation level was high in both years, interseeded crimson clover plots still had the lowest weed biomass of any of the interseeded forage plus cultivation treatments. This indicates that crimson clover was able to effectively compete with weeds even at high weed infestation levels. This could be partly attributed to the early germination and rapid early growth of crimson clover which would give it an early competetive advantage over the emerging weeds. Another contributing factor could be the high seeding rate used in this experiment, which would have provided higher forage densities which could compete effectively with the developing weed populations. These results agree with findings of Nelson et al. (1991) who reported that crimson clover was a promising legume with respect to cover density and ability to suppress weed populations.

In both years at the Macdonald site, hairy vetch was not able to suppress the weed population. This could be attributed to its slow growth at the begining of the season, when weeds were becoming established, and contradicts several previous studies indicating the effectiveness of hairy vetch as a weed suppressor (e.g. Johnson et al., 1993). Hoffman et al. (1993) reported that untreated hairy vetch reduced weed biomass 96 and 58% in the two-years of their study. On the other hand, Teasdale (1993) found that hairy vetch did not provide effective weed control, as it retained soil moisture during the dry season, thereby allowing the weeds to establish more easily than in drier plots without a cover.

4.3.4. Corn Yield:

In 1994, corn yields were not affected by any of the treatments (unpublished data) presumeably due to the decrease in competition for moisture and the beneficial effects of the legumes from the previous year. In 1993 at Macdonald, the interseeded cover crops did not affect corn yields (unpublished data) except for crimson clover which was competetive enough to reduce the grain yields by 19% relative to the weeded controls. In 1993 at l'Assomption, all the cover crop treatments reduced corn yields compared with the weeded controls, but the yields of these treatments were higher than the weedy treatment, except for Persian clover and strawberry clover which were not different from the weedy control. Averaged accross both sites and years, a significant negative correlation (-0.29) was found between corn yield and weed biomass, indicating a decline in grain yield as weed biomass increased. Weed biomass in plots interseeeded with crimson clover and alfalfa were negativly correlated with corn garin yield.

4.4. Conclusion:

The weed population at l'Assomption was greater than at Macdonald, resulting in a better establishment of the cover crops at the latter site. Several cultivations prior to the seeding of the cover crops controled 70 and 80% of the weeds present at Macdonald, and l'Assomption respectively; the inclusion of the cover crops provided an additional 10% control. Due to the intense weed infestation at l'Assomption, there was no additional weed control from the cover crops. Higher weed populations were observed on the corn rows compared with between the rows. Crimson clover was able to establish well under weedy conditions, and was able to suppress more weeds than any of the other treatments. Hairy vetch was not very competitive, as it provided good cover but only later in the growing season. In the presence of adequate moisture, corn grain yields were not affected by any of the treatments. The crimson clover treatment reduced corn yield by 19% due to its highly competitive ability and rapid establishment.

Table 4.1. Total monthly precepitation and average temperature recorded at Macdonald and l'Assomption during 1993 and 1994 and 30 year averages.

Year	May	June	July	August	September	Total	May	June	July	August	September
			Precepitation mm Macdonald						Temperature °C Macdonald		
1993	79.1	74.8	94.6	57.2	119.2	424.9	13.3	17.6	21.4	20.5	13.9
1994	148	194	61.3	99.9	105.5	607.8	12.1	18.9	21.3	18.0	14.3
Average *	70.6	88.3	89.7	92.6	97.9	439.1	13.1	18.1	21.1	19.8	14.7
			l'Assomption						l'Assomption		
1993	95.6	74.2	75.4	95.6	89.1	429.9	12.7	17.4	20.7	20.3	13.6
1994	93.8	285.9	122.8	67.8	121.6	691.9	11.8	19.3	21.0	19.2	14.7
Average *	72.5	87.0	84.5	94.4	84.6	423.0	12.3	17.5	20.2	18.8	13.8

* 30-yr averages

Table 4.2. Seeding rates of the interseeded forage legumes and grasses in 1993 and 1994 at Macdonald and l'Assopmtion

Cover crop names			
Common Name	Cultivar	Latin Name	Seeding rate kg ha^{-1}
Fall rye	Prima	*Secale cereale* L.	110
Hairy vetch	Canada No. 1	*Vicia villosa* Roth	30
Red clover + ryegrass	Khun + Marshall	*Trifolium pratense* L. + *Lolium multiflorum* L.	10 + 8
White clover + ryegrass	Ladino + Marshall	*Trifolium repens* L. + *Lolium multiflorum* L.	7 + 8
Subterranean clover	Northam	*Trifolium subterraneum* L.	12
Yellow sweet clover	Canada No. 1	*Melilotus officinale* L.	7
Black medic	Canada No. 1	*Medicago lupulina* L.	15
Persian clover	Canada No. 1	*Trifolium resupinatum* L.	10
Strawberry clover	Salina	*Trifolium fragiferum* L.	7
Crimson clover	Canada No.	*Trifolium incarnatum* L.	22
Alfalfa	Nitro	*Medicago sativa* L.	12
Berseem clover	Canada No. 1	*Trifolium alexandrinum* L.	20

Table 4.3. Weed population and biomass as affected by interseeded forage legumes and grasses at Macdonald 1993

Forage Species	On row		Between row		Total	Total
	number	weight (g)	number	weight (g)	number	weight (g)
Fall Rye	3.9 b	15.9 b	1.9 bc	1.3 bc	6.4 b	18.4 bc
Hairy vetch	5.3 b	1.0 b	4.3 b	5.3 b	10.5 b	23.7 bc
Red Clover + Rye grass	5.3 b	15.5 b	2.1 bc	1.2 bc	9.2 b	21.3 bc
White Clover + Rye grass	7.9 ab	25.5 ab	3.9 b	3.1 bc	13.3 b	32.4 b
Subterranean clover	5.3 b	19.8 b	2.4 bc	1.5 bc	8.3 b	22.0 bc
Yellow sweet clover	3.1 bc	7.7 bcd	2.1 bc	0.8 bc	5.7 b	9.6 cde
Medicago lupelina	7.3 ab	24.2 ab	1.8 bc	3.5 bc	10.0 b	29.6 bc
Persian clover	4.0 b	12.0 bc	2.4 bc	1.1 bc	6.9 b	13.2 bcd
Strawberry Clover	3.9 bc	13.6 b	3.9 b	2.0 bc	8.6 b	17.2 bc
Crimson clover	2.7 bc	10.3 bc	2.4 bc	1.5 bc	5.7 b	12.0 bcd
Alfalfa	3.3 bc	13.0 bc	3.1 bc	1.4 bc	6.9 b	15.7 bc
Berseem clover	6.8 ab	23.5 ab	1.8 bc	0.3 c	9.3 b	23.8 bc
Hand weeded Control	3.5 bc	1.5 cd	2.1 bc	0.4 c	6.0 b	1.8 de
Chemically Weeded Control	0.5 bc	0.5 b	0.5 c	0.1 c	1.2 c	1.8 e
Weedy control	13.2 a	45.7 a	12.5 a	41.5 a	26.5 a	89.8 a

Values within the same column, followed by the same letter are not different by an ANOVA Waller-Duncan test ($p \leq .05$)

Table 4: Weed population and biomass as affected by interseeded forage legumes and grasses at Macdonald 1994

Forage Species	On row		Between row		Total	Total
	number	weight (g)	number	weight (g)	number	weight (g)
Fall Rye	5.1 bc	3.5 bc	8.2 abc	4.8 b	13.5 bc	8.7 b
Hairy vetch	2.5 bc	1.3 bc	11.8 ab	4.1 b	14.4 bc	5.4 b
Red Clover + Rye grass	6.6 b	2.2 bc	2.8 c	1.7 bc	9.8 bcd	3.9 bc
White Clover + Rye grass	6.2 b	5.0 b	9.0 abc	2.9 bc	15.8 b	8.2 b
Subterranean clover	3.5 bc	1.2 c	6.7 bc	2.4 bc	10.7 bcd	3.8 bc
Yellow sweet clover	5.6 bc	4.8 b	6.6 bc	2.6 bc	12.7 b	7.7 b
Cultivation	6.5 b	3.3 bc	8.7 abc	4.4 b	15.5 bcd	8.4 b
Persian clover	2.7 bc	1.2 bc	6.9 bc	3.9 b	10.1 bcd	5.4 bc
Strawberry Clover	5.4 bc	2.2 bc	7.3 bc	5.4 ab	13.0 b	8.2 b
Crimson clover	3.5 bc	1.0 bc	3.0 c	2.2 bc	7.0 bcd	3.3 bc
Alfalfa	3.9 bc	0.8 bc	4.1 bc	2.5 bc	8.0 bcd	3.6 bc
Berseem clover	3.7 bc	1.8 bc	4.7 bc	1.8 bc	8.6 bcd	3.8 bc
Hand weeded Control	2.6 bc	0.4 c	2.3 c	0.2 c	5.3 bcd	0.6 c
Chemically Weeded Control	1.8 c	0.8 bc	2.4 c	1.2 bc	4.1 cd	1.8 bc
Weeded control	19.9 a	16.1 a	16.9 a	10.7 a	37.5 a	27.5 a

Values within the same column, followed by the same letter are not different by an ANOVA Waller-Duncan test ($p \leq .05$)

Table 4.5. Weed population and biomass as affected by interseeded forage legumes and grasses at l'Assomption 1993

Forage Species	On row		Between row		Total	Total
	number	weight (g)	number	weight (g)	number	weight (g)
Fall Rye	14.1 b	26.9 bc	12.2 bc	6.4 bcd	27.2 b	35.3 bc
Hairy vetch	26.6 ab	55.2 ab	10.9 bcd	7.4 bcd	38.1 b	63.8 abc
Red Clover + Rye grass	28.9 ab	56.8 ab	9.0 bcd	3.3 cd	39.4 b	62.1 abc
White Clover + Rye grass	27.5 ab	46.6 abc	14.7 bc	12.0 bc	44.5 b	58.8 abc
Subterranean clover	24.6 ab	48.4 abc	10.7 bcd	7.2 bcd	36.2 b	56.2 abc
Yellow sweet clover	27.5 ab	64.1 a	14.9 bc	7.3 bcd	43.3 b	74.7 ab
Medicago lupelina	14.5 b	45.9 abc	9.4 bcd	6.7 bcd	24.4 b	54.4 abc
Persian clover	15.9 b	32.0 abc	15.3 bc	15.2 b	33.4 b	55.3 abc
Strawberry Clover	28.9 ab	45.6 abc	11.9 bc	9.1 bcd	41.4 b	55.5 abc
Crimson clover	21.0 b	27.6 cd	18.8 ab	7.6 bcd	42.0 b	27.8 c
Alfalfa	25.7 ab	35.9 abc	12.3 bc	4.9 bcd	37.9 b	41.8 bc
Berseem clover	24.6 ab	57.1 ab	7.4 cd	2.2 d	33.1 b	60.2 abc
Hand weeded Control	15.0 b	4.0 d	9.4 bcd	3.5 cd	25.3 b	7.7 d
Chemically Weeded Control	3.5 c	4.1 d	3.1 d	2.0 d	6.6 c	6.1 d
Weeded control	39.6 a	40.7 abc	33.5 a	43.7 a	79.4 a	86.4 a

Values within the same column, followed by the same letter are not different by an ANOVAWaller-Duncan test ($p \leq .05$)

Table 4. 6. Weed population and biomass as affected by interseeded forage legumes and grasses at l'Assomption 1994

Forage Species	On row		Between row		Total	Total
	number	weight (g)	number	weight (g)	number	weight (g)
Fall Rye	33.9 ab	11.2 b	28.2 b	4.0 bc	63.7 ab	16.4 bc
Hairy vetch	27.8 ab	8.1 b	58.0 ab	5.3 bc	86.3 ab	14.6 bcd
Red Clover + Rye grass	23.7 ab	10.3 b	26.1 b	3.1 bc	50.9 ab	14.5 bcd
White Clover + Rye grass	81.4 a	6.0 b	40.6 ab	5.3 bc	124.9 a	12.1 bcd
Subterranean clover	26.7 ab	12.6 b	41.9 ab	5.0 bc	66.7 ab	21.9 b
Yellow sweet clover	22.8 ab	7.8 b	57.9 ab	5.5 bc	82.3 ab	14.5 bcd
Cultivation	25.4 ab	6.1 b	78.5 a	5.4 bc	106.6 a	12.2 bcd
Persian clover	24.4 ab	4.2 b	30.1 ab	6.7 b	56.5 ab	11.8 bcd
Strawberry Clover	38.9 ab	8.7 b	65.3 ab	4.7 bc	105.9 a	14.4 bcd
Crimson clover	21.0 ab	7.0 b	28.9 ab	4.1 bc	50.6 ab	11.6 bcd
Alfalfa	73.2 a	7.4 b	50.8 ab	4.3 bc	139.2 a	12.0 bcd
Berseem clover	15.2 ab	10.3 b	56.4 ab	5.2 bc	76.9 ab	17.4 bcd
Hand weeded Control	32.4 ab	3.6 b	30.4 ab	1.8 c	64.0 ab	5.7 d
Chemically Weeded Control	4.6 b	2.8 b	4.0 c	4.0 c	8.8 b	6.9 d
Weeded control	35.3 ab	32.4 a	52.8 ab	27.0 a	92.4 a	60.7 a

Values within the same column, followed by the same letter are not different by an ANOVA Waller-Duncan test ($p \leq .05$)

Table 4.7. Correlation between weed biomass in individual interseeded plots and corn grain yield.

	Correlation Coefficient
Fall Rye	-0.240
Hairy Vetch	0.214
Red clover + Rye grass	0.177
White clover + Rye grass	0.060
Subterranean clover	0.131
Yellow sweet clover	0.241
Cultivated control	-0.125
Persian clover	-0.03
Strawberry clover	0.06
Crimson clover	-0.447*
Alfalfa	0.127
Berseem clover	-0.460*
Hand weeded control	0.127
Chemically weeded control	-0.184
Weedy control	-0.574*

*Significant at the 0.05 level of probability

Preface to Chapter 5

This chapter is part of a manuscript by Abdin et al. to be submitted to the Agronomy Journal for publication in 1997. The format has been changed to conform to a consistent format within this thesis. All literature cited is listed at the end of the thesis. Each table or figure for chapter 5 is presented at the end of this chapter.

After demonstrating the effects of different forage legumes and grasses on grain corn, and the effectiveness of some of them as weed suppressers, we tested the potential use and performance of different forage legumes and grasses and the effects of their seeding date on their dry matter production. A range of forages were tested in terms of their ability to produce high dry matter under competition for light and nutrients. This dry matter would eventually be incorporated into the soil and contribute to improving and maintaining soil quality.

Chapter 5

POTENTIAL USE OF FORAGE LEGUMES AND GRASSES AS COVER CROPS IN GRAIN CORN IN EASTERN CANADA

5.0. Abstract:

Field experiments were conducted at two Québec locations in 1993 and 1994 to evaluate the potential use of forage legumes and grasses as interseeds in corn in eastern Canada. Twelve forage species were evaluated. Fall rye (*Secale cereal* L.), hairy vetch (*Vicia villosa* Roth), a mixture of red clover (*Trifolium pratense* L.) and ryegrass (*Lolium multiflorum* Lam), a mixture of white clover (*Trifolium repens* L.) and ryegrass, subterranean clover (*Trifolium subterraneum* L.), yellow sweet clover (*Melilotus officinalis* Lam.), black medic (*Medicago lupulina* L.), Persian clover (*Trifolium resupinatum* L.), strawberry clover (*Trifolium fragiferum* L.), crimson clover (*Trifolium incarnatum* L.), annual alfalfa (*Medicago sativa* L.), and berseem clover (*Trifolium alexandrinum* L.) were seeded at two planting dates, 10 and 20 days after corn emergence. The control treatments were hand weeding, chemical weeding and non weeded. Early seeded forages established better and had higher biomass accumulation than the late seeded ones. In the presence of larger weed populations, the interseeded forages did not develop well due to the competition with the weeds. At Macdonald crimson clover provided good soil cover while Persian clover, fall rye and alfalfa provided

relatively little cover. Strawberry clover and hairy vetch did not provide early ground cover due to their late development in the season. Forage mixtures of red or white clover and rye grass established well and achieved high populations at the end of the growing season. Fall rye provided good early ground cover but senesced by the middle of the season. The better establishment and early germination of crimson clover caused a 19% reduction in corn grain yield in 1993. In 1994, none of the cover crops caused a reduction in corn yield.

5.1. Introduction:

The benefits of cover crops have long been known. According to Pieters (1927) Chinese writers indicated the importance of legume cover crops in increasing the yield of following crops more than 2000 years ago. Recent studies support this. Decker et al. (1994) reported an increase in corn grain yield following cover crops. Bollero and Bullock (1994) reported an increase in corn grain yield following a hairy vetch cover crop. In addition to increased yield of the following crop, cover crops can provide soil cover which reduces soil erosion (Wall et al., 1991). Several studies have also indicated an increase in soil organic matter due to incorporating cover crops (Holderbaum, et al. 1990; Brown et al. 1993), as well as an improvement in the soil physical properties (McVay et al. 1989; Latif et al., 1992). Due to the beneficial effects of legume cover crops it has been a common practice to rotate a legume crop with a grain crop so as to maintain the soil, while increasing the yield of the succeeding crop.

Although alternating a legume cover crop with a grain crop provides the above benefits, farmers have to sacrifice the grain yield in the cover crop production year. A possible alternative is to interseed the cover crops between corn rows so as to maintain the soil without sacrificing the grain crop. A good cover crop should give adequate ground cover throughout the growing season without interfering with the main crop. Several cover crops have been used as interseeds between corn rows. Because of its rapid early season growth, crimson clover has been successfully cover cropped resulting in dry matter yields of up to 6.7 Mg ha^{-1} (Hargrove 1986). Scott et. al. (1987) reported that interseeding annual rye grass (*Lolium multiflorum* Lam.) and medium red clover (*Trifolium pratense* L.) resulted in high dry matter production and provided adequate ground cover without affecting corn yield. Stemann et al. (1993) have also shown that interseeding rye grass (*Lolium multiflorum* Lam.) in corn prevented soil erosion and produced corn yields higher than those of the controls. In another study by Tychon et al. (1992), rye grass (*Lolium perenne*) developed well under a maize canopy and served to protect the soil from erosion. Exner and Cruse (1993) found that interseeded alfalfa and red clover had established better and produced more cover than either red clover or alsike clover. In Ontario, Wall et al. (1991) reported that the interseeding of red clover in corn rows provided soil cover so as to protect the soil from erosion without affecting maize silage yield. On the other hand, Claude et al. (1993), in Québec, reported that interseeding red clover in corn had limited potential as a cover crop due to weed competition. Stute and Posner (1993) reported that interseeded hairy vetch gave the highest dry matter yield when compared with other leguminous cover crops used in the experiment. Subterranean clover (*Trifolium subterraneum*) has been shown to be effective as a cover crop to control weeds in corn (Ilnicki and Enache, 1992).

Time of seeding of the cover crop is considered crucial if acceptable yield from the main crop is to be achieved. Nordquist and Wicks (1974) reported a grain yield reduction of up to 3 t ha^{-1} when alfalfa was seeded simultaneously with corn. Exner and Cruise (1993) reported a decrease in corn grain yields when the interseeded forage legumes were sown at the same time as the corn. Scott et al. (1987) delayed planting of the inteseeded forages until the corn was 15-30 cm high and reported no grain yield reduction during the first year. Later planting dates were less effective in controlling weeds (Palada et. al., 1982).

Smith et al. (1987) pointed out that although it is possible to grow legume cover crops in a wide range of environments, restrictions imposed by environmental conditions such as temperature and water availability limits their potential to grow fully and serve as cover for the summer crop. Since cover crops differ in their vigor and tolerance to stressful environmental conditions, a comprehensive search for possible forage species that are adapted to a particular location should be carried out in order to determine their suitability as intercrops in corn.

The objectives of this study were (i) to conduct an evaluation of forage legumes and grasses potentially useful for interseeding in grain corn in eastern Canada and (ii) to determine if an earlier seeding date than suggested in the literature from more southern locations would be appropriate in eastern Canada.

5.2. Materials and Methods:

An experiment to evaluate the cover crop potential of 12 forage legumes and grasses was conducted in 1993 and 1994 at two Québec sites, the E. A. Lods Agronomy Research Center, McGill University, Macdonald Campus, Québec, Canada, and the Agriculture and Agri-Foods Canada Research Farm at l'Assomption, Québec, Canada. The two sites are approximately 80 km apart. The soil at the Macdonald site was a mixture of Chateauguay clay (fine loamy, mixed, nonacid, frigid, Hapludalf) and St. Bernard clay (fine loamy, mixed, nonacid, frigid, Eutrochrept). At l'Assomption the soil type was a Soulange silt loam (fine-silty, mixed, nonacid, frigid Humaquept).

Soil tests prior to planting showed that the soil at the Macdonald campus site had a pH of 5.5, thus five t ha^{-1} of agricultural limestone was applied to raise the pH. Two hundred and ten, and 95 kg ha^{-1} of 19-8-15 (N-P-K) were added to the soil through the corn seeder at the Macdonald and l'Assomption sites, respectively. Additional fertilizer was broadcast immediately prior to planting to achieve the recommended rates of 180, 37 and 100 kg ha^{-1} of N, P, and K, respectively. The soil was harrowed 7 days before planting after which the lime and fertilizer were broadcast and disked in to obtain a smooth seedbed. The Macdonald site was fallow the year before starting the experiment, while the l'Assomption site had been in pasture the previous year.

The experiment was conducted in a split-plot arrangement with 4 replications at each site. The two factors under study were the time of forage planting and the type of forage species. The whole plots comprised two ground cover planting dates: 10 and 20 days after corn emergence. Scott and Burt (1985) suggested that interseeded forage species be seeded when corn is 15 to 43 cm high, corresponding approximately to 20 days after seeding. However, in northern locations this leaves a shorter period for subsequent cover crop development and earlier seeding would be desirable. Thus, we also tested a 10 day after corn emergence, when the corn was approximately 11 cm tall. The subplots were twelve forage species or mixtures and three controls. The seeding rates used at all site years are given in Table 5.2. Legumes were inoculated prior to planting with the appropriate commercial inoculant. In 1993 the three controls were a hand weeded, chemically weeded and unweeded treatments. In 1994, a control consisting of mechanical weeding with a Rabewerk cultivator (Rabewerk Machinerie Agricole, St.-Césaire, QC, Canada) was added for each planting date. This control replaced the black medic cover crop treatment of the previous year. The corn hybrid Pioneer 3921 was planted at a rate of 80,000 plants ha^{-1}. The main plot size was 15 m by 21 m and the subplot size was 3 by 7 m. Each sub plot consisted of 4 corn rows planted 75 cm apart with 16.4 cm between plants of the same row.

Corn was planted on the 11 and 13 May in 1993 at l'Assomption and Macdonald, respectively. In 1994, corn was planted on 11 and 14 May at Macdonald and l'Assomption, respectively. The corn seeders used were a, John Deere planter (model Max Emerge2 7200) at l'Assomption and a Gaspardo planter (SP 510, Pordenone, Italy) at Macdonald. They were both adjusted to give a planting density of 80,000 seeds ha^{-1}. The 1994 experiment was planted at the same site as 1993 and treatment plots were planted in the same location each year. Cultivation was conducted weekly until forage seeding, with the Rabewerk cultivator (a rigid tine cultivator with goose foot). A mixture of Dual metolachlor [2-chloro-N-(2-ethyle-6-methylephenyl)-N-(2-methoxy-1-methylethyl) acetamide] and atrazine [6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2,4-diamine] were applied preemergence at a rate of 1.9 and 1.0 L ha^{-1} respectively, to the chemically treated control plots using a bicycle-wheel plot sprayer. Forages were hand broadcast over the plots at their respective densities. Average monthly rainfall and temperatures for each site year plus the thirty year averages for each month are given in Table 5.1. Corn was harvested at maturity, during the second week of October in both years.

Forage samples were collected at sampling times; during the second week of August (first sampling), and prior to the corn harvest in mid October (second sampling). Quadrats of 0.5 m by 0.2 m were randomly placed in the plots, the forages were hand cut just above the soil surface, identified, counted, placed in paper bags, and dried to a constant weight at 70°C for biomass determination.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1985) was used for analysis of variance of all data. Probabilities equal to or less than 0.05 were considered significant for main effects and interactions. The least significant difference (LSD) test was used to separate differences between treatment means if analysis of variance indicated the presence of such differences (Steel and Torrie, 1980).

5.3. Results and Discussion:

Weather data showed higher levels of precipitation in 1994 than 1993 at both sites. They were also higher than the 30 year average at both locations. Temperatures were almost the same in both years at the two sites and were comparable to the 30 year averages (Table 5.1).

5.3.1. Seeding Date:

In 1993, mean forage biomass in early seeded treatments was 32% and 17.5% higher than those obtained from the late seeded treatments at the Macdonald and l'Assomption sites, respectively. In 1994 at Macdonald, yield of the early seeded forages was 21% higher than late seeded ones. This is in agreement with results obtained by Scott et al. (1987) where interseeded legumes and grasses produced good stands and ground cover when they were seeded at the same time, relative to the corn crop, as the early seeding in our experiment. Stute and Posner (1993) reported a decrease in dry matter of legume cover crops interseeded in corn as their sowing date was delayed.

In both years and locations, the mixture of rye grass and white clover produced the highest yields for the second planting date, indicating that the lower temperatures encountered by the early seeded material were probably a limiting factor in the establishment of white clover. Except for the rye grass/white clover mix, early seeded plots established better than late seeded ones.

5.3.2. Forage ground cover:

In both years at Macdonald early seeded treatments were able to provide an earlier soil cover than the late seeded cover crops (Tables 5.4 and 5.6). At l'Assomption there were no differences in ground cover, as the forages did not develop well due to severe weed competition. In 1993 at both the Macdonald and l'Assomption sites, crimson clover provided the greatest ground cover, which was comparable to those provided by Persian clover, fall rye, and annual alfalfa at Macdonald, and fall rye and annual alfalfa at l'Assomption.

In 1993 at the Macdonald site, strawberry clover provided the least ground cover due to its poor germination and late development. At l'Assomption, hairy vetch, white cover plus rye grass, subterranean clover, yellow sweet clover and strawberry clover provided the least ground cover.

In 1994 at the Macdonald site, fall rye and crimson clover provided the greatest soil cover as they were the earliest to develop. At l'Assomption, red clover plus rye grass, annual alfalfa, crimson clover, white clover plus rye grass, and Persian clover established well and provided good ground cover. Hairy vetch did not develop well early in the season and therefore provided poor ground cover.

5.3.3. Forage population and biomass:

In 1993 at the Macdonald site, there were no differences between the average forage population of early and late seeded treatments; however, forage biomass from the early seeded treatments was higher than that of the late seeded ones. Early seeded forages likely had more time to accumulate biomass than the late seeded forages. In 1994 at the l'Assomption site there were no differences between early and late seeding forage for population or biomass. At Macdonald, late seeded forages had higher average populations than the early seeded ones but there were no differences in biomass accumulation. This could be attributed to better germination conditions later in the season leading to a higher population which offset the time advantage of early seeding, at least for biomass production. In 1994 at both sites, red clover plus rye grass achieved the highest population. They were greater than those of fall rye, hairy vetch, subterranean clover and yellow sweet clover at both sites, in addition to strawberry clover at the Macdonald site.

In 1994 at the Macdonald site, crimson clover produced the most biomass. Strawberry clover, yellow sweet clover, subterranean clover, and hairy vetch produced the least biomass. At the l'Assomption site, crimson clover and red clover plus rye grass produced high biomass values that were not different from those of white clover plus rye grass, Persian clover, and berseem clover. Yellow sweet clover, hairy vetch, and strawberry clover were among the lowest yielding interseeded forages at that harvest date. Fall rye had a lower biomass yield, probably due to senescence in the period leading up to corn harvest.

5.3.4. Location:

The l'Assomption site in 1993 was generally more weedy than the Macdonald site. This presented an obstacle to the establishment of the forages at that location. High weed populations have been reported to hinder the establishment of interseeded forages. Claude et al. (1993) reported difficulty in the establishment of interseeded red clover in Québec unless weed populations were kept under tight control. Averaged over both seeding dates, forage biomass yields were higher at the l'Assomption site than at the Macdonald site (Table 5.9). This was mainly due to the severe competition from the pre-exiting weeds. Mean forage yield at l'Assomption in 1994 was higher due to better weed control, as an additional cultivation was performed prior to forage seeding, thereby reducing the weed population at the time of this seeding. Higher levels of precipitation at l'Assomption (Table 5.1) could also explain the better establishment of some of the forages at this sight than at Macdonald.

5.3.5. Forage Species:

Fall rye was the first cover crop to germinate at either the late or early planting dates (visual observation), it provided adequate and early season cover (Tables, 5.4, 5.6 and 5.7). However, by the end of July, plants started to senesce. Yields reported in this experiment were collected from dried fall rye plants. Despite its early senescence, fall rye provided good soil cover.

Hairy vetch did not provide adequate ground cover early in the season. Most of the biomass developed by this forage species was accumulated later in the season. In addition, hairy vetch has a climbing growth habit, so that plants tended to grow vertically on the corn rather than horizontally leading to provision of less soil cover.

The red and white clover/rye grass mixes had high above ground biomasses in all four site years (Table 5.9). Scott et al. (1987) reported similar results with a mixture of rye grass and medium red clover. Although not significant, under our experimental and environmental conditions, the red clover/rye grass mix tended to provide more ground cover and biomass than the white clover/rye grass.

In 1993 and 1994 at the Macdonald site, subterranean clover had comparatively lower biomass yields (Table 5.9) and ground covers (Tables, 5.5 and 5.7). This could be attributed to the growth habit of subterranean clover, as it grows closer to the ground without providing enough elevated leaf area to compete for light with weeds. This contradicts results by Enache and Ilnicki (1990) who reported a high efficiency of subterranean clover in controlling weed populations.

Strawberry clover biomass developed later in the season, due to both late germination and slow growth, so it did not provide adequate cover at the beginning of the season (Tables 5.5 and 5.7).

Crimson clover was one of the promising interseeded forage legumes included in this experiment. Its high biomass and good early season ground cover (60%) was consistent over all four site-years. However, the better establishment and early germination of crimson clover caused a 19% reduction in corn grain yield in 1993.

At the l'Assomption site in 1994, berseem clover had greater dry matter yields than alfalfa, indicating the greater suitability of berseem clover than alfalfa for use in interseed systems. However, in the presence of high weed populations berseem clover failed to establish and was out yielded by alfalfa. (Table 5.9)

5.3.6. Grain Yield:

Corn grain yield was not affected by the time of forage seeding in any site-year (chapter 3).

In 1993 at the Macdonald site crimson clover caused a 19% reduction in grain yield when compared with the chemically and hand weeded controls. This could be attributed to its rapid establishment and early development between the corn rows, such that it competed strongly with the growing corn for water and nutrients. Plots interseeded with yellow sweet clover had a lower grain yield than those from subterranean clover. The rest of the forages did not increase or decrease corn yields when compared with the weeded control at that site. All interseeded treatments in 1993 at the Macdonald site had higher yields than those obtained from the weedy control.

In 1994 at Macdonald, interseeded forages did not affect corn yields when compared with any of the controls. This may have been due to the availability of adequate moisture that year. In 1993 at l'Assomption, all the interseeded treatments reduced corn grain relative to the weeded controls, but had higher yields than the weedy control, except for Persian clover, strawberry clover, and crimson clover which had yields that were not different from the weedy control.

5.4. Conclusions:

The early seeded forages yielded more biomass and provided better ground cover than the late seeded ones. Crimson clover established well in all four site years. Strawberry clover did not provide good soil cover due to its late germination. The mixtures of red clover or white clover and rye grass had high populations at the end of the growing season. The biomass yield of fall rye was low due to its senescence in the period leading up to corn harvest. Hairy vetch did not provide adequate ground cover early in the season. The red or white clover and ryegrass mixes had high above ground biomasses in all four site years. The better establishment and early germination of crimson clover caused a reduction in corn grain yields in 1993.

Table 5.1. Total monthly precipitation and average temperature recorded at Macdonald and l'Assomption during 1993 and 1994 and 30 year averages.

Year	May	June	July	August	September	Total	May	June	July	August	September
			Precipitation mm Macdonald						Temperature °C Macdonald		
1993	79.1	74.8	94.6	57.2	119.2	424.9	13.3	17.6	21.4	20.5	13.9
1994	148	194	61.3	99.9	105.5	607.8	12.1	18.9	21.3	18.0	14.3
Average *	70.6	88.3	89.7	92.6	97.9	439.1	13.1	18.1	21.1	19.8	14.7
			l'Assomption						l'Assomption		
1993	95.6	74.2	75.4	95.6	89.1	429.9	12.7	17.4	20.7	20.3	13.6
1994	93.8	285.9	122.8	67.8	121.6	691.9	11.8	19.3	21.0	19.2	14.7
Average *	72.5	87.0	84.5	94.4	84.6	423.0	12.3	17.5	20.2	18.8	13.8

* 30-yr averages

Table 5.2. Seeding rates of the interseeded forage legumes and grasses in 1993 and 1994 at Macdonald and l'Assomption.

Cover crop names			
Common Name	Cultivar	Latin Name	Seeding rate kg ha^{-1}
Fall rye	Prima	*Secale cereale* L.	110
Hairy vetch	Canada No. 1	*Vicia villosa* Roth	30
Red clover + ryegrass	Khun + Marshall	*Trifolium pratense* L. + *Lolium multiflorum* L.	10 + 8
White clover + ryegrass	Ladino + Marshall	*Trifolium repens* L. + *Lolium multiflorum* L.	7 + 8
Subterranean clover	Northam	*Trifolium subterraneum* L.	12
Yellow sweet clover	Canada No. 1	*Melilotus officinale* L.	7
Black medic	Canada No. 1	*Medicago lupulina* L.	15
Persian clover	Canada No. 1	*Trifolium resupinatum* L.	10
Strawberry clover	Salina	*Trifolium fragiferum* L.	7
Crimson clover	Canada No.	*Trifolium incarnatum* L.	22
Alfalfa	Nitro	*Medicago sativa* L.	12
Berseem clover	Canada No. 1	*Trifolium alexandrinum* L.	20

Table 5.3. Main effect of forage species on forage biomass at the Macdonald and l'Assomption sites, first sampling date.

Forage species	Forage biomass ($gm\ d\ m^{-2}$)			
	Macdonald		l'Assomption	
	1993	1994	1993	1994
Fall Rye	5.1 cdef	3.6 b	6.3 ab	1.0 f
Hairy vetch	6.0 cde	2.1 b	6.6 a	2.6 ef
Red clover + Rye grass	9.1 ab	9.0 a	6.9 a	9.3 ab
White clover + Rye grass	5.1 cdef	6.6 a	5.7 abc	7.4 abc
Subterranean clover	2.3 g	2.7 b	1.2 bdc	6.4 bcd
Yellow sweet clover	3.8 defg	2.0 b	2.6 abcd	2.2 ef
Black medic	2.8 fg	----	2.3 abcd	----
Persian clover	6.2 cd	6.7 a	0.5 d	3.7 edf
Strawberry Clover	3.3 fg	3.5 b	3.1 abcd	4.6 edf
Crimson clover	9.4 a	6.6 a	5.9 abc	10.6 a
Annual alfalfa	3.5 efg	2.6 b	2.8 abcd	1.6 ef
Berseem clover	6.6 bc	7.7 a	0.7 cd	4.3 cdef
Hand weeded Control	-----	-----	-----	-----
Chemically Weeded Control	-----	-----	-----	-----
Weedy control	-----	-----	-----	-----

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.4. Main effect of seeding date on percent ground cover by forages in 1993.

Forage seeding	Percent ground cover	
	Macdonald	l'Assomption
Early	47.6 a	20.9 a
Late	21.3 b	18.7 a

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.5. Main effect of forage species on percent ground cover by forage species in 1993.

Forage species	Percent ground cover (%)	
	Macdonald	l'Assomption
Fall Rye	54.3 a	39.6 a
Hairy vetch	22.5 d	6.0 fg
Red Clover + Rye grass	36.6 bc	16.6 bcde
White Clover + Rye grass	33.1 bc	14.8 defg
Subterranean clover	18.2 d	9.5 efg
Yellow sweet clover	28.7 cd	10.0 defg
Black medic	27.5 cd	21.4 bcd
Persian clover	44.3 ab	16.0 cdef
Strawberry Clover	7.3 e	6.7 fg
Crimson clover	55.6 a	41.0 a
Annual alfalfa	44.3 ab	28.3 ab
Berseem clover	40.6 b	26.6 bc

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.6. Main effect of seeding date on percent ground cover by forages in 1994.

Forage seeding	Ground cover (%)	
	Macdonald	l'Assomption
Early	30.5 a	29.2 a
Late	9.6 b	25.1 a

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.7. Main effect of forage species on percent ground cover by forage species in 1994; first sampling.

Forage species	Ground cover (%)	
	Macdonald	l'Assomption
Fall Rye	43.6 a	19.6 bc
Hairy vetch	12.3 de	7.5 c
Red Clover + Rye grass	22.1 bc	46.1 a
White Clover + Rye grass	15.6 cd	28.8 ab
Subterranean clover	11.5 de	18.1 bc
Yellow sweet clover	8.1 e	19.5 bc
Persian clover	20.7 bc	30.5 ab
Strawberry Clover	6.5 e	15.8 bc
Crimson clover	35.7 a	43.8 a
Annual alfalfa	24.6 b	27.1 b
Berseem clover	21.2 bc	44.0 a

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.8: Main effects of forage species on population and biomass at the Macdonald site in 1993; second sampling.

Forage Species	Population (Plants dm^{-2})	Biomass (g dm^{-2})
Fall Rye	31.4 cde	15.0 a
Hairy vetch	9.0 f	2.0 d
Red Clover + Rye grass	97.6 a	10.3 ab
White Clover + Rye grass	100.2 abc	9.1 ab
Subterranean clover	19.1 de	5.0 bc
Yellow sweet clover	13.7 ef	1.8 cd
Black medic	60.3 ab	5.6 ab
Persian clover	40.8 bcd	7.9 ab
Strawberry Clover	20.2 de	1.0 d
Crimson clover	37.0 bcd	8.6 a
Annual alfalfa	45.6 abc	5.3 ab
Berseem clover	54.7 ab	7.2 ab

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.9. Main effects of forage species on population and biomass at the Macdonald and l'Assomption sites 1994; second sampling.

Forage Species	Macdonald		l'Assomption	
	Population (Plants dm^{-2})	Biomass (g dm^{-2})	Population (Plants dm^{-2})	Biomass (g dm^{-2})
Fall Rye	9.8 d	3.0 bcd	14.6 de	3.2 ef
Hairy vetch	4.8 d	2.0 bcde	6.1 e	1.2 f
Red Clover + Rye grass	73.2 a	3.3 bc	94.3 a	8.6 a
White Clover + Rye grass	38.7 ab	3.1 b	67.5 a	7.4 abc
Subterranean clover	12.7 cd	1.3 de	22.0 bc	4.1 bcde
Yellow sweet clover	9.5 d	1.5 cde	12.4 cd	1.2 f
Persian clover	57.1 ab	3.8 bc	57.1 a	4.6 abcd
Strawberry Clover	29.6 bc	0.4 e	64.5 a	2.4 def
Crimson clover	30.6 ab	8.8 a	51.0 ab	8.7 a
Annual alfalfa	34.5 ab	3.6 bc	44.1 ab	3.0 cde
Berseem clover	30.3 ab	2.7 bcd	61.6 a	6.3 ab

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Table 5.10. Main effect of seeding date on interseeded forage population and biomass at the Macdonald and l'Assomption sites in 1993 and 1994; second sampling.

Forage seeding date	1993		1994			
	Macdonald		Macdonald		l'Assomption	
	Population Plant d m^{-2}	Biomass g d m^{-2}	Population Plant d m^{-2}	Biomass g d m^{-2}	Population Plant d m^{-2}	Biomass g d m^{-2}
Early	28.6 a	6.5 a	12.6 b	2.8 a	42.3 a	3.4 a
Late	28.5 a	2.3 b	19.9 a	1.5 a	15.0 a	4.3 a

Values within the same column, followed by the same letter are not different by an ANOVA protected LSD test ($p \leq 0.05$).

Preface to Chapter 6

This chapter is part of a manuscript by Abdin et al. to be submitted to the Crop Science journal for publication in 1997. The format has been changed to conform to a consistent format within this thesis. All literature cited is listed at the end of the thesis. Each table or figure for chapter 6 is presented at the end of this chapter.

In an attempt to study within plant competition for resources, this chapter deals with the development of a stem injection system through which solutions could be administered to legumes to study physiological processes that occur during competition. Soybean has been used as a model leguminous plant in this experiment, largely because of its rapid growth and thick stem.

Chapter 6

EFFECT OF SUCROSE SUPPLEMENTATION BY STEM INJECTION ON THE DEVELOPMENT OF SOYBEAN PLANTS

6.0. Abstract:

Stem injection methods have been developed for cereal plants over the past half decade. These methods allow researchers to administer solutions to cereal plants to study their effects on plant physiology. However, little work has been done to extend this technique to non-cereals. An experiment was conducted to test an injection technique that would be suitable for soybean plants, and to study the effect of long term injection of sucrose on the growth of soybean plants. An injection set-up, comprised of a supporting stand and a fluid injection system was established. Pressure was applied to the plunger of a 5 mL syringe using ceramic bricks in order to force test solutions into the plants. Solutions of 0, 150, and 300 g sucrose L^{-1} were injected into soybean plants for eight weeks starting at the seedling VC stage. Distilled water had the highest uptake rate, followed by the 150, and then the 300 g sucrose L^{-1} solutions. The overall average uptake during the injection period was 77.3 mL. Average sucrose uptake values were 11.8 and 13.5 g per plant for 150 and 300 g sucrose L^{-1} treatments. This represented approximately 65% of the total dry weight of the plant. Sucrose infusion increased leaf area and pod number relative to the control treated plants. Nodule numbers were lower for sucrose injected treatments, however; their dry weights were higher for control. Above soil dry matter was higher for plants injected with 300 g sucrose L^{-1} than those injected with water. The injection system

was able to administer concentrated solutions into soybean plants for most of their growth and development period. The sucrose supplementation had positive effects on soybean growth but probably suppressed photosynthesis.

6.1. Introduction:

Numerous studies have attempted to elucidate the effect of elevated availability of carbon on the growth of soybean plants (*Glycine max* [L.] Merr.) (Rogers et al. 1984; Mulchi et al. 1995; Sicher et al. 1995). In most of these studies, extra carbon was supplied as carbon dioxide. In most cases carbon dioxide was used as, being a gas normally taken up by plant leaves, it is easily administered. Prior and Rogers (1995) reported an increase in the total leaf area and dry weight of soybean plants exposed to elevated carbon dioxide concentrations. Carbon dioxide supplementation has also been shown to increase plant photosynthesis, and biomass accumulation (Baker et. al., 1989; Allen, 1991), and yield (Rogers et al. 1984). Root growth of soybean plants was also enhanced when plants were supplied with elevated levels of carbon dioxide (Reinert and Ho, 1995).

Continuous exposure to elevated levels of carbon dioxide does not mean continuously elevated photosynthetic rates due to partial stomatal closure (Mckee et al., 1995; Demothes, 1996), and at very high carbon dioxide levels plants will become carbon dioxide saturated. Very high levels of carbon dioxide may cause stomatal closure, and may lead to a reduction in photosynthesis (Hicklenton and Jolliffe, 1980). Diffusion of carbon dioxide into plant cells is mainly controlled through stomates. Low levels of carbon dioxide stimulate the opening of stomates, while high carbon dioxide concentrations in the leaves can cause the stomates to close partially, allowing plants to minimize water loss. Chen et al. (1995) reported a reduction in the stomatal conductance of soybean plants when exposed to elevated levels of carbon dioxide. When stomates are completely closed, the presence of external carbon dioxide would have no effect. Stomatal movement is also affected by external factors such as humidity, water potential, temperature, and light (Fay and Knapp, 1995). Thus, these factors can alter the rate of diffusion of carbon dioxide into plant tissues.

The principal storage products of carbon dioxide fixation are sucrose and starch (Bassham, 1965; Goldschmidt et al., 1992) Sucrose is readily translocated through the phloem (Patrick and Offler, 1995). Sicher et al. (1995) reported an increase in the levels of starch and sucrose when soybean plants were placed under higher carbon dioxide concentrations. Production of sucrose would, therefore, be dependent upon factors affecting photosynthesis and carbon dioxide diffusion.

One way to overcome the effects of plant reactions to carbon dioxide levels and external influences on carbon dioxide absorption, is to directly supply the plant with sucrose. Traditional methods of supplying substances such as sucrose via leaves or roots, are only appropriate for solutions that can be readily taken up by these tissues. In studies requiring addition of substances that are not easily, taken up using current methods, newer methods of delivery should be sought. Early studies by Spoehr (1942) demonstrated the ability to supply albino corn plants, that are incapable of producing their own sugars through photosynthesis, with sucrose through the cut ends of their leaves. However, this method only allowed sucrose addition for a short period of time and the amount of the solution absorbed was limited. Grabau et al. (1986), succeeded in injecting methionine into intact soybean plants through a stem perfusion technique using an intravenous feeding system; plants were able to take up an average of 51.2 mL per plant from seed development until physiological maturity. Using the same technique, Schon and Blevins (1987) were able to infuse mineral salts of boron and calcium into intact soybean plants. Boyle et al. (1991a) designed a stem infusion system for corn using small cavities in the stem made at multiple sites. This system was capa of delivering exogenous supplies of dilute solutions at 5 to 10 mL h^{-1} per site (Boyle et al. 1991a) and a total of 84 and 117 milliliters per plant of concentrated sucrose solution in two experiments, respectively (Boyle et al. 1991b). Ma and Smith (1992) developed a perfusion system to supply nitrogenous solutions into the peduncle of barley (*Hordeum vulgare* L. cv. Leger) plants. This system was capable of supplying up to 68 mL of the solution over 20 days, which increased grain nitrogen concentration by up to 40% compared with the non-perfused or distilled water-perfused controls. Using the peduncle perfusion technique Ma et al., (1994) did not find any change in the carbon or nitrogen contents of grain or non grain tissues of barley or wheat (*Triticum aestivum* L. emend. Thell.) when injected with sucrose or growth regulators, but found increased amino acid concentrations in both wheat and barley when injected with nitrogen, while sucrose injection increased lysine concentration in wheat only (Ma et al., 1995). Using the perfusion technique developed by Boyle et al. (1991a), Zinselmeier et al. (1995) reported an elimination of drought stress related grain yield reductions by sucrose infusion. A technique was recently reported by Zhou and

Smith (1996) in which it was feasible to supply sucrose and water solutions to field grown corn plants through syringe needles, using ceramic bricks as the source of pressure. The average plant intake was 5.1 mL per day per plant with an average total intake of 163 mL per plant.

Most the recent studies involving the testing of new perfusion/injection techniques to externally supply liquid substances into intact plant tissues have involved cereal plants, and mainly corn and barley. However, in both grass systems the movement of the sheath relative to culm tissues during vegetative development has meant that an infusion or injection system could only be established after stem elongation was largely completed (about the time when reproductive development begins). This limitation would not exist for legume plants, making extension of a chronic injection system to plants of this type of additional interest.

The objective of this study was to develop a perfusion technique that is suitable for soybean plants and which allows the injection of large amounts of liquid substances over a long time period. A modification of the perfusion system developed by Zhou and Smith (1996) was tested for its suitability and efficiency, and the effects of an increased supply of reduced carbon on soybean growth and development were measured.

6.2. Materials and Methods:

A greenhouse experiment was conducted in 1995 (Plant Science Department of McGill University, Ste. Anne de Bellevue, QC, Canada). Soybean (cv 'Maple Glen') plants were inoculated with a commercial innoculant of *Bradyrhizobium japonicum* (Nitragin, LiphaTech, Milwaukee, Wisconsin, USA) and planted in trays filled with a 1:1 mixture of sand and Turface (Applied Industrial Materials Corp., Illinois, USA). Seedlings were left to grow in the trays until they attained the VC stage [unifoliate leaves were unfolded sufficiently that the edges were not touching (Fehr and Caviness, 1977)]. Plants in the trays were watered as necessary. Vigorous seedlings were selected from the trays, and transplanted into 15.5 cm diameter and 15 cm deep pots, and containing the same rooting medium as the transplanting trays. A 16-h photoperiod was maintained using supplemental lighting from high pressure sodium lamps. Temperature was maintained between 22 and 25 °C. Relative humidity was approximately 75%. After being transplanted into the pots soybean plants were watered regularly using a modified Hoagland's solution (Hoagland and Arnon, 1950), in which $CaNO_3$ and KNO_3 were replaced with $CaCl_2$, K_2HPO_4, and KH_2PO_4 to provide a nitrogen free solution.

An injection set up was established (modified from Zhou and Smith, 1996) prior to the start of the experiment (Fig 6.1a and b). This was comprised of two main parts; (i) a supporting stand, and (ii) a fluid injection system. The injection stand consisted of a 30 X 31 cm plywood base of 1.22 cm thickness. Two circular metal bases attached near the back end of the wooden base (20 cm apart) with wood screws, and two threaded metal pipes 59 cm in length and with a 1.22 cm outside diameter were threaded vertically onto the metal bases. A hose clamp was tightened around each of the pipes at a distance of 30 cm above the wooden base, and a 23 cm X 13 cm wooden platform rested on the hose clamps. Two holes 1.4 cm in diameter, and 25 cm apart, were made in the wooden platform. At mid distance between these two holes, a third hole of 1.0 cm was drilled. The center hole supported a 5 mL syringe. The injection tubing consisted of a 35 cm long flexible plastic tubing (Tygon i.d. 0.8 mm, o.d. 2.4 mm) that was connected at one end to a standard disposable 18-gauge 1 1/2 needle (Becton Dickinson and Company, Franklin Lakes, NJ), and at the other end to a 25-gauge 3/4 vacutainer needle (Vacutainer, Becton Dickinson and Company, Rutherford, NJ) that was modified prior to its use. The vacutainer needle was bent to an angle of approximately 60° and the original tubing that came with the vacutainer system, was removed, only the needle and the supporting "wings" were kept intact. The initial tubing was replaced with the heavier Tygon as initial testing revealed that the original vacutainer tubing ruptured under high pressure. The needles attached to each end of the tygon tubing were sealed in place with epoxy resin glue.

The injection apparatus was established two days after transplanting. Using masking tape, a triangular cup shape was formed around the stem of soybean seedlings about 1 cm above the soil surface. Using the winged end of the injection tubing, the 25-gauge needle was inserted into the stem of a soybean plant, so that at least half the needle length was inside the stem. After needle insertion, the cup was filled with fluid latex (Vultex, General Latex Canada, Candiac, QC) and was left to dry for a period of 5 days after the VC stage, in order to ensure a proper seal at the injection site. After drying, the pots were placed on the injection stand, and the other end of the injection tubing was connected to a 5 mL-syringe, that contained about 2 mL from the solution pertinent to the treatment under study. In order to replace the air present in the Tygon tubing with the injected solution, the syringe piston was pulled back, drawing plant sap into the tubing and replacing all the air. The syringe piston was then gradually released, so that liquid could flow freely into the tube. The syringe was then disconnected from the tubing, and filled completely (5 mL) with the treatment solution, placed in its designated place on the injection stand, and reconnected with the injection tubing. After assembling the injection system, pressure was applied to the syringe by placing a ceramic brick (approximately 2.7 kg each) on top of the syringe plunger. The bricks were the standard (22.5 by 8.5 by 7 cm) three hole construction type. One brick was added each day until reasonable flow rates were achieved. This never required more than 4 bricks. Approximately three times the number of experimental units required were initially set up, so as to achieve the required number of working systems with no leaks or obstructions.

The experimental design was a randomized complete block with four replicates. One replicate was established each week for four weeks, providing replication in time. The treatments consisted of a distilled water injected control, and two sucrose concentrations, 150 and 300 g L^{-1}.

The amount of injected solution was monitored regularly, and the syringe barrels were refilled as necessary. The injected plants were examined daily to make sure that there were no leaks. Plants were harvested at maturity. Pods from each treatment were counted and oven dried at 90°C to a constant weight. Leaf area was determined using an area measuring system (Delta-T Devices Ltd., Burwell, Cambridge, England).

Chlorophyll fluorescence measurements were recorded at flowering using a Morgan CF-1000 chlorophyll fluorescence measurement system (Morgan Scientific Inc., Andover, MA.). Three measurements were taken from each pot. One cuvette per plant was placed on the uppermost fully expanded leaf. The cuvette was left for about 10 minutes on each leaflet so that the leaflets were acclimatized to darkness. The optical probe was then inserted into the cuvette and a reading was taken and used to determine the Fo (non-variable fluorescence), the Fm (maximal fluorescence), the Fv (variable fluorescence), and the ratio of Fv:Fm, which is a measure of the photochemical efficiency of photosystem II. Readings from the 3 samples were averaged to calculate the soybean chlorophyll fluorescence for that treatment. Plant height was measured from the soil surface to the tip of the stem. Nodules from each treatment were counted and dried at 90 °C in a forced air oven.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1985) was used for analysis of variance of all data. Probabilities equal to or less than 0.05 were considered significant. The least significant difference (LSD) test was used to separate differences between treatment means if analysis of variance indicated the presence of such differences (Steel and Torrie, 1980).

6.3. Results and Discussion:

6.3.1. Rate of infusion:

Insertion of the vacutainer needle into the plants did not seem to cause any large scale reaction by them, and the solutions were absorbed freely by the plant for an average of 3 days, after which the rate of uptake slowed, probably due to the build up of a callus tissue by the plant in response to wounding at the site of injection. Ma et al. (1994) reported that the dead tissue resulting from the injection process reduced the amount of solution administered to corn plants, causing limited solution uptake. However, in our case this situation was overcome by pulling the piston barrel forward and backward several times to clear the blockage.

A clear pattern was observed concerning the volumes of injected solutions. The distilled water had the highest average uptake rate, followed by the 150 g L^{-1}, and then the 300 g L^{-1} sucrose solutions (Fig. 6.2 and 6.3). This was probably due to the higher osmotic potential of the infused sucrose solutions, which make it more difficult for soybean plants to absorb them. Similar results were reported by Zhou and Smith (1996), where corn plants had higher uptake rates for distilled water than concentrated sucrose solutions. Ma et al. (1994) also noted the greater resistance of barley tissue to the entry of concentrated solutions such as sucrose, than to water. The overall average uptake rate was 77.3 mL (1.3 mL d^{-1}). Schon and Blevins (1987), using a different injection technique, managed to administer 18.5 mL $week^{-1}$ of amino acid solution to soybean plants.

Converting the injection volume to grams of added sucrose, average sucrose uptake values were 11.8 and 13.5 g for the 150 and 300 g L^{-1} solutions, respectively (Fig. 6.12). Zhou and Smith (1996) reported an uptake of 17.7 and 40.9 g L^{-1} for 150 and 300 g L^{-1} sucrose solutions, respectively for injected corn plants. Boyle et al. (1991) were able to infuse approximately 15 g of sucrose per corn plant. In our case, the amount of added sucrose represents 64 and 65% of the total dry weight of soybean plants receiving the 150 and 300 g L^{-1} solutions, respectively. Zhou and Smith (1996) reported injection of sucrose equivalent to 30% of the dry matter gain during the injection period due to injection of sucrose into corn plants.

Less pressure (indicated by the number of bricks) was needed to inject the least concentrated solutions. Usually 2-3 bricks were used in the distilled water treatment, compared with 4 bricks for the sucrose treatments. For the 300 g L^{-1} sucrose treatment, uptake was slow during the first 3 to 4 days, as the bricks were added, one at a time during this period so as to prevent sudden pressure increases that could cause the system to leak. Until four bricks were added, the pressure was not sufficient to cause good flow. Zhou and Smith (1996) also reported slower uptake of the concentrated sugar solutions. They overcame this by adding more pressure (bricks). In our case, the amount of solution administered to plants each day varied from 1 to 3 mL.

A decline in the rate of sucrose uptake, especially with the 300 g L^{-1} solution, was noted as the soybean plants developed (Fig. 6.2). Several interpretations to this phenomena have been proposed. Zhou and Smith (1996) postulated that the decline in sucrose uptake over time was due to production of callus or tyloses, possibly using some of the injected sugars, in response to the insertion wound (Trapley et al., 1994). However, Fourtan-pour and Smith (1996) observed the same phenomenon in a perfusion system where the needle tips rested in the tissue free space inside the peduncles of barley plants. Considering the long period during which the soybean plants were subjected to injection, a build up of callus structures could have contributed to the slower uptake of sugars later in the growing period.

6.3.2. Effects on soybean plants:

Plants infused with the 300 g sucrose L^{-1} solution flowered 3 to 4 days earlier than plants infused with either 150 g L^{-1} or distilled water (data not shown). Leaf number and leaf area of plants infused with sucrose were higher than those of plants infused with distilled water (Figs 6.4 and 6.5), with the 150 g L^{-1} sucrose solution resulting in the numerically highest levels of theses variables. Similar results were reported by Prior and Rogers (1995) who found an increase in the leaf area of soybean plants supplied with elevated levels of carbon dioxide.

Plants injected with distilled water had lower pod numbers than those injected with 300 g sucrose L^{-1}, however they were not different from plants injected with 150 g sucrose L^{-1} (Fig. 6.10). Prior and Rogers (1995) observed that plants grown under elevated carbon dioxide levels produced more pods by maturity than those receiving ambient carbon dioxide levels. Similar results were reported by Rogers et al. (1986). Shoot dry weights of soybean plants were higher for sucrose injected plants than for those receiving distilled water. Rogers et al. (1993) reported an increase in biomass production when soybean plants were in air enriched with carbon dioxide.

Nodule numbers were lower in plants receiving sucrose compared with those receiving distilled water, and there was no difference in nodule number between the two sucrose concentrations (Fig. 6.6). However, the dry weight of nodules from the 150 g sucrose L^{-1} infused treatments were higher than those from plants infused with distilled water (Fig.

6.7), suggesting that plants compensated for the lower nodule number by producing larger nodules (5.2 mg nodule $^{-1}$).

Chlorophyll fluorescence, measured as the ratio of Fv:Fm, showed lower values for plants injected with distilled water and 300 g L^{-1} sucrose, than those receiving 150 g L^{-1} sucrose (Fig. 6.13). This would indicate that plants receiving 300 g L^{-1} sucrose are under stress compared with those receiving a lower concentration, or have poorly functioning photosynthetic systems due to high levels of sucrose availability.

Plant dry matter was higher for plants injected with the 300 g L^{-1} sucrose than those injected with distilled water (Fig. 6.7), but they were not different from plants injected with the 150 g L^{-1} sucrose. Soybean plant heights were higher for both sucrose injected treatments than for those injected with water (Fig. 6.8).

Although sucrose injected plants had greater leaf areas than those injected with distilled water, the amount of accumulated dry matter due to photosynthesis (g plant dry weight g^{-1} injected sucrose) in those treatments (7.5 and 8.7 g per plant) was lower than the control (11.4 g plant $^{-1}$), suggesting lower photosynthetic activity level for the sucrose injected plants.

6.3.3. Conclusions:

The injection system developed was able to inject substantial amounts of concentrated solutions of sucrose into the stems of soybean plants. The rate of injection declined at the end of the injection period, which may have been due to callus tissue production at the injection site. Soybean plants injected with sucrose had higher dry matter accumulations, and lower photosynthetic rates than those injected with distilled water.

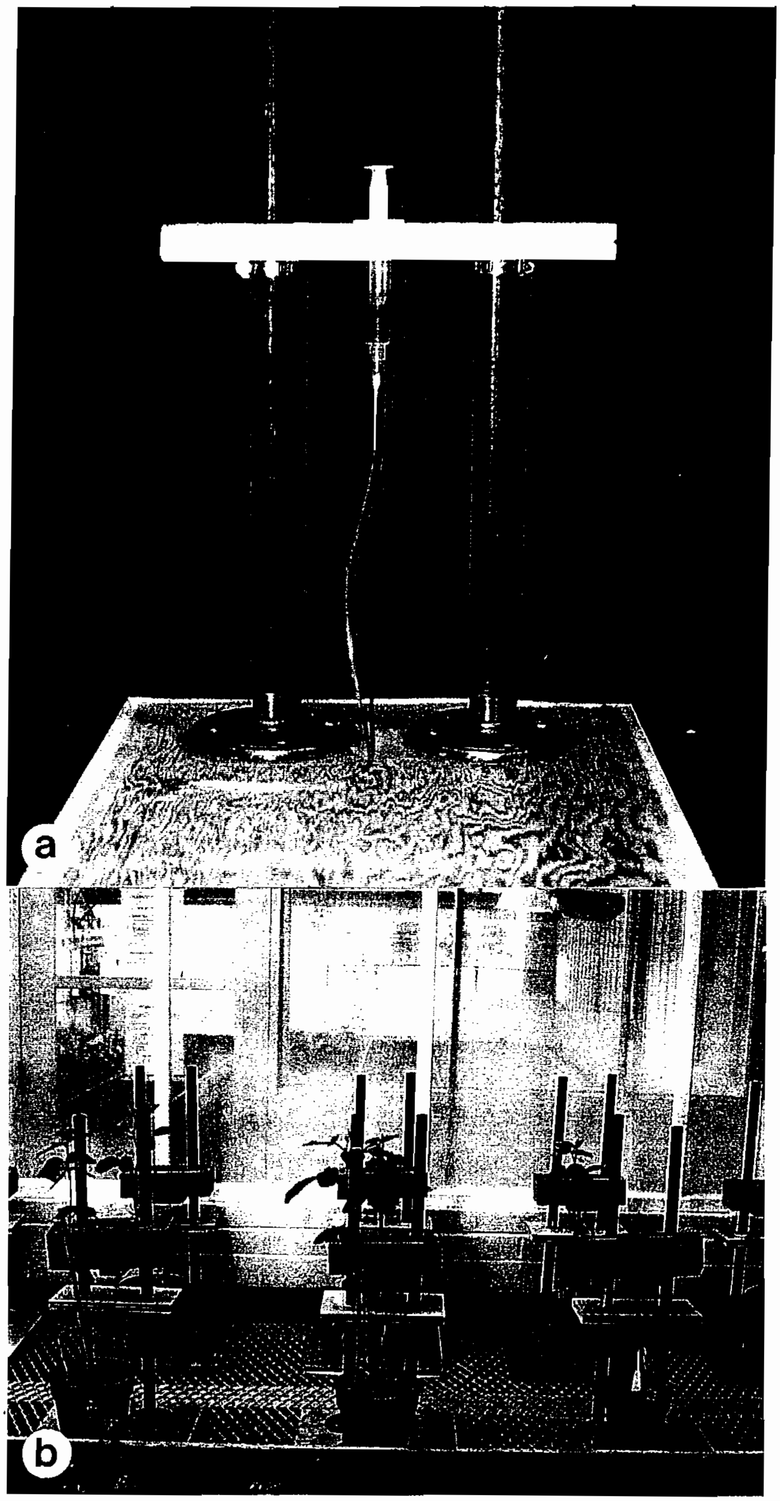


Fig. 6.1. Injection system (a) and the system in use in greenhouse (b).

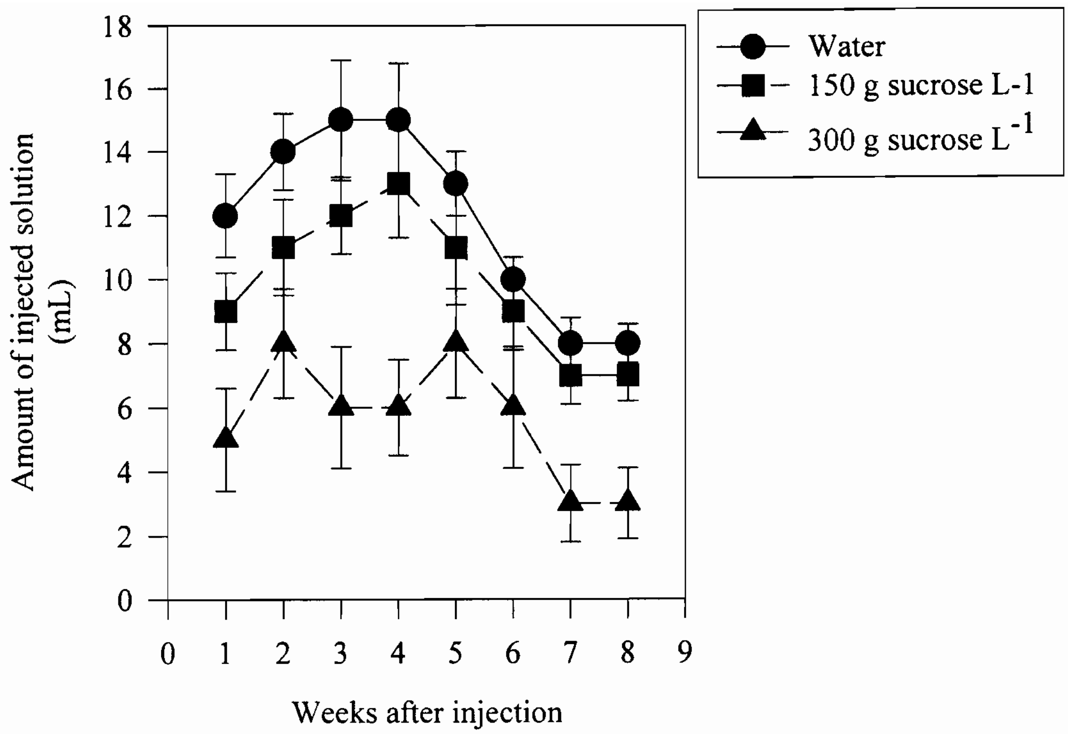


Fig 6.2. Average weekly uptake of solutions during an 8 week period.

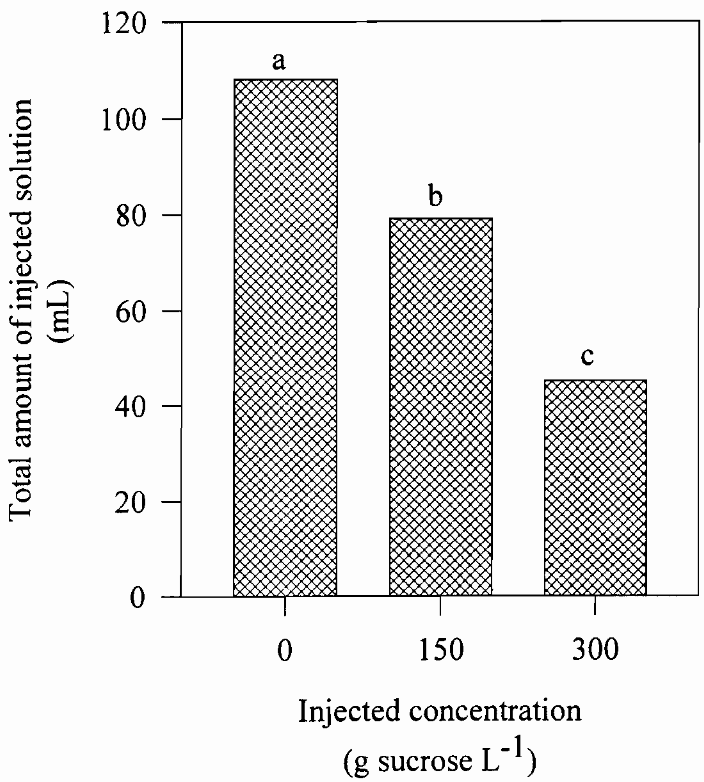


Fig. 6.3. Total amount of injected solution during an 8 week injection period.

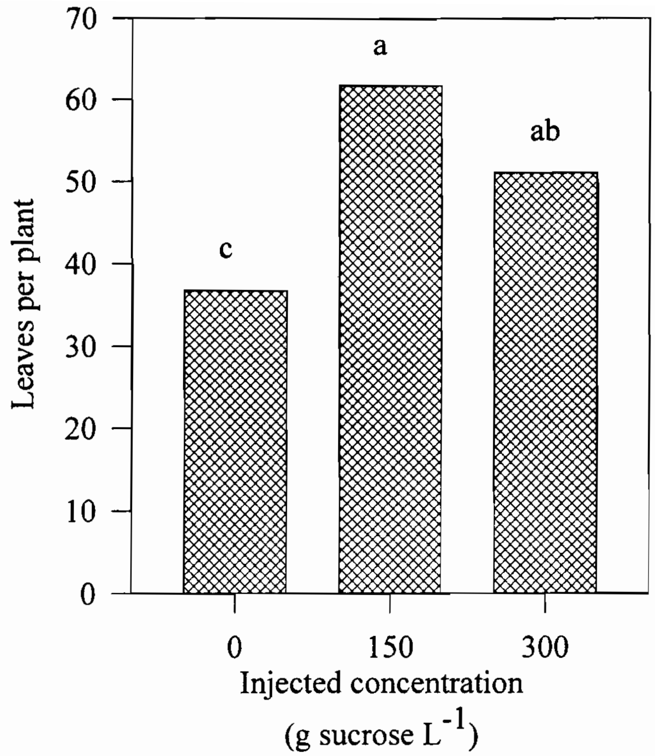


Fig.6.4. Effect of sucrose concentration on the number of soybean leaves.

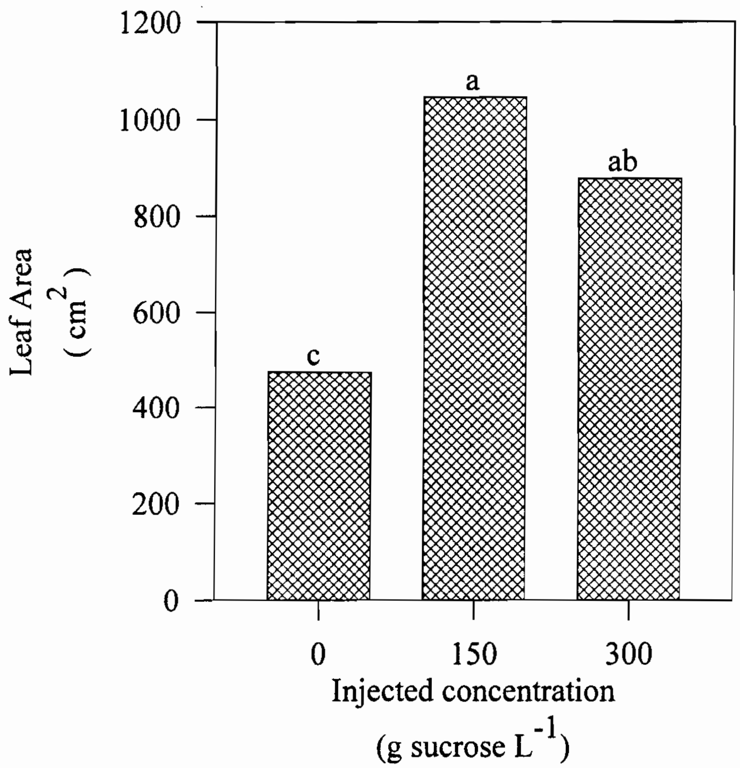


Fig. 6.5. Effect of sucrose concentration on soybean leaf area.

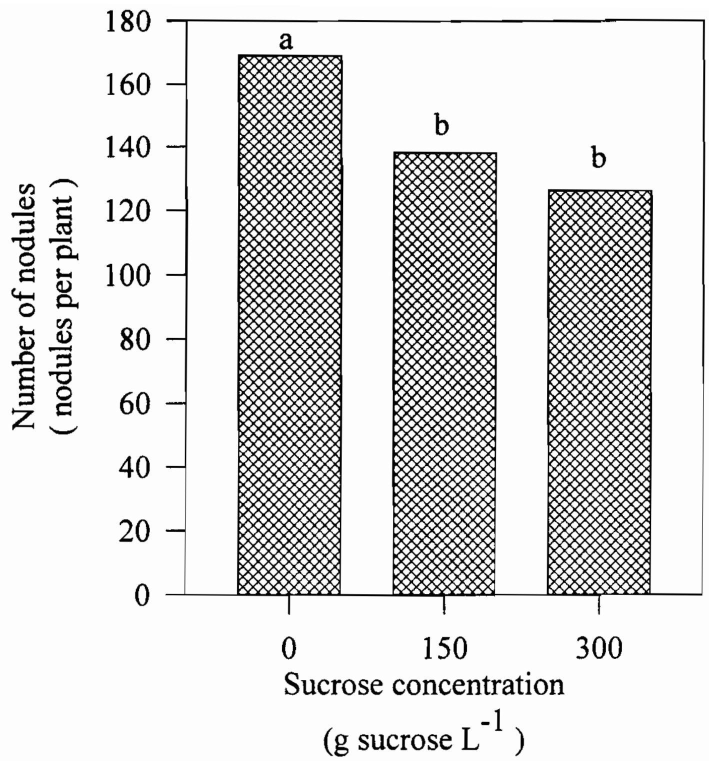


Fig.6.6. Effect of sucrose concentration on the number of soybean nodules.

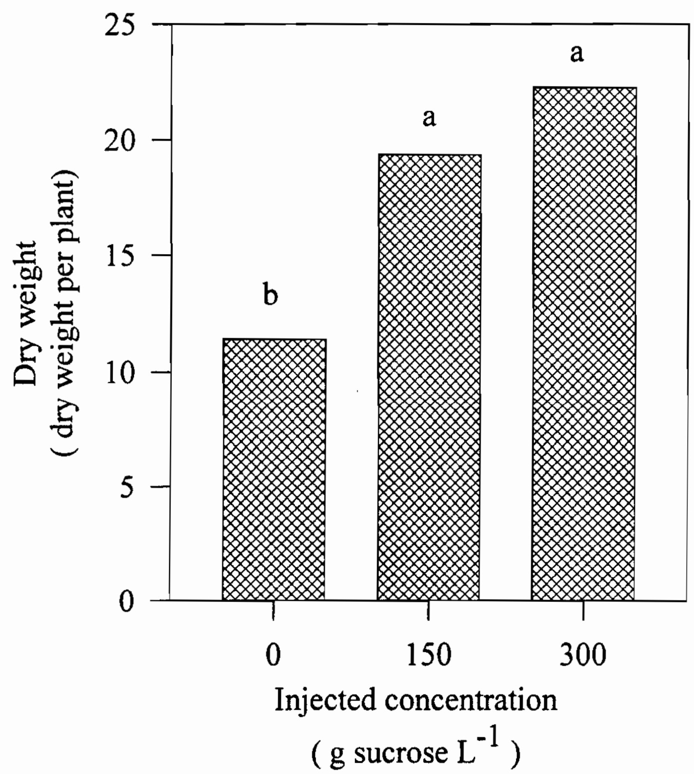


Fig.6.7. Effect of sucrose concentration on soybean dry weight.

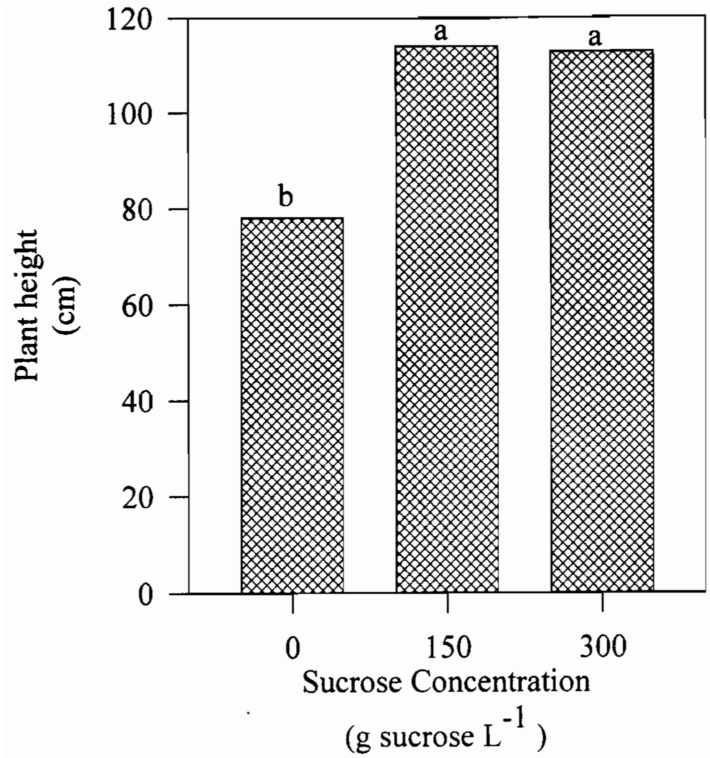


Fig.6.8. Effect of sucrose concentration on soybean plant height.

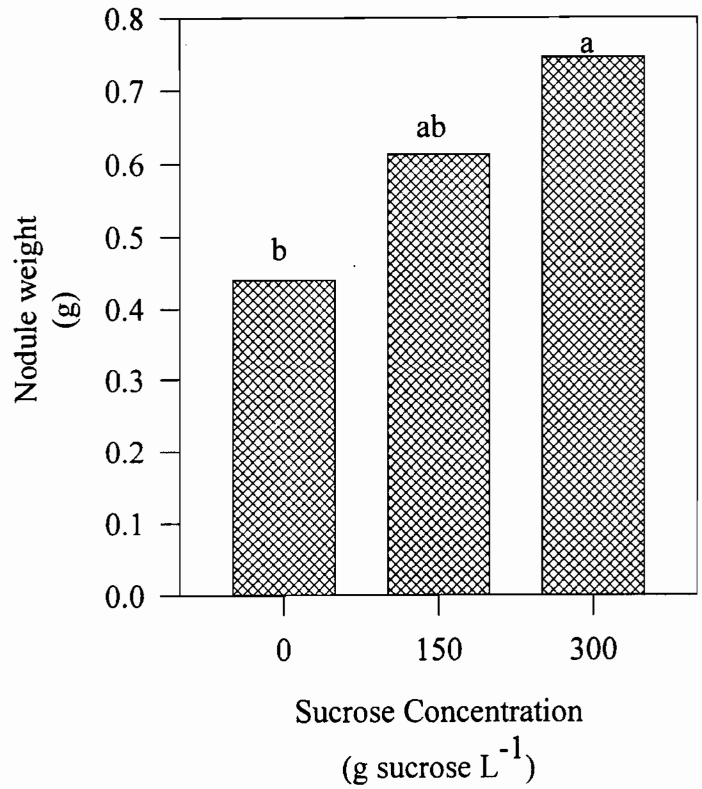


Fig. 6.9. Effect of sucrose concentration on soybean nodule weight.

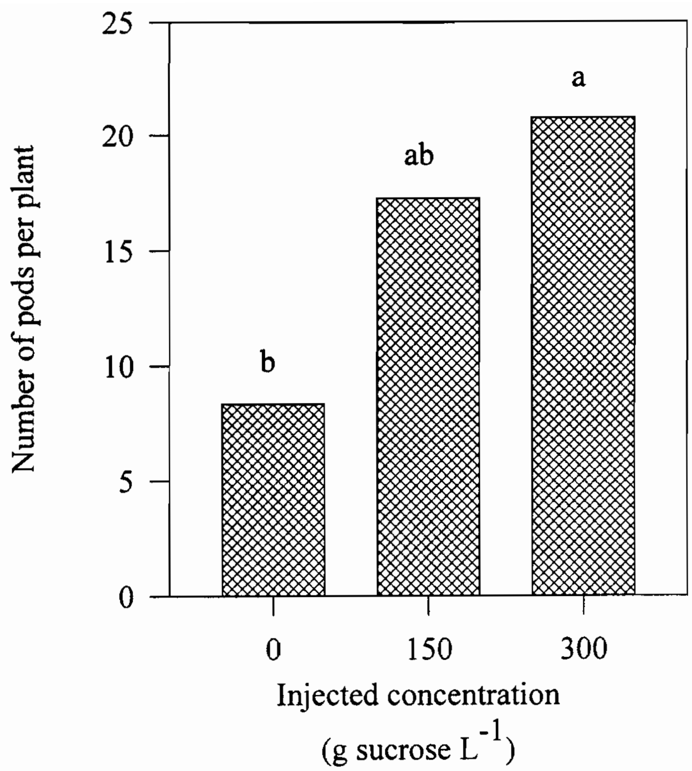


Fig. 6.10. Effect of sucrose concentration on the number of soybean pods.

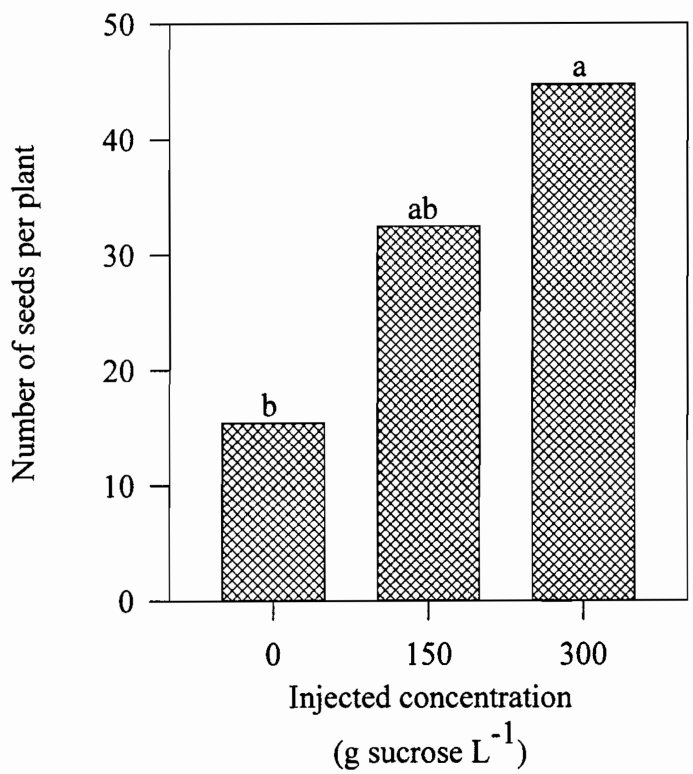


Fig. 6.11. Effect of sucrose concentration on number of seeds.

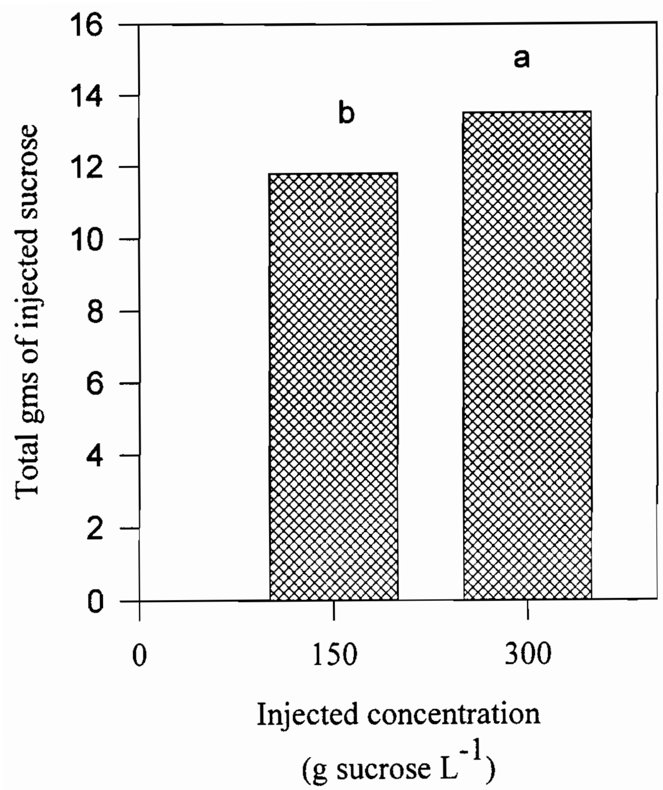


Fig. 6.12. Total amount of injected sucrose.

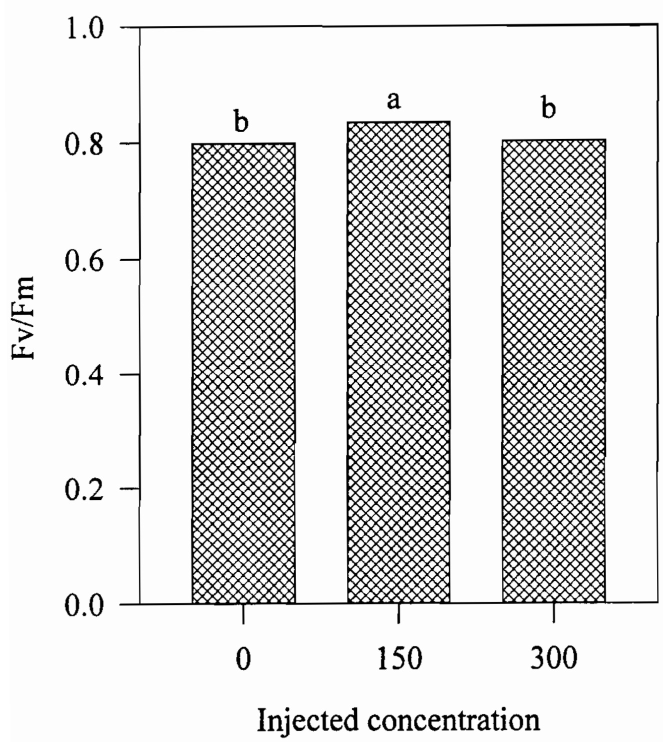


Fig. 6.13. Effect of sucrose concentration on fluorescence values of soybean plants.

Preface to Chapter 7

This chapter is part of a manuscript by Abdin et al. to be submitted to the Crop Science journal for publication in 1997. The format has been changed to conform to a consistent format within this thesis. All literature cited is listed at the end of the thesis. Each table or figure for chapter 7 is presented at the end of this chapter.

After demonstrating the suitability and success of the injection technique. We tested the effects of carbon and nitrogen supplementation to plants placed under shade, a condition that simulates shaded plants in the field in a polyculture system. In this chapter, the effects of supplying sucrose and nitrogen or a combination of both on plants placed under different levels of shade was investigated.

Chapter 7

EFFECT OF SUCROSE AND NITROGEN SUPPLEMENTATION BY STEM INJECTION ON THE DEVELOPMENT OF SOYBEAN PLANTS GROWN UNDER VARYING LEVELS OF SHADING.

7.0. Abstract:

Light can often be a limiting factor in the growth and development of crops grown in polyculture systems. Previous studies have shown decreases in the growth and development of plants under shade, which was mainly due to a decrease in carbon assimilation by these plants, as a result of reduced light interception. Reduced light intensities can also reduce the ability of plants to take up or fix nitrogen, especially in a competitive situation. A greenhouse experiment was conducted to test the effects of increased carbon (sucrose) and nitrogen (urea) supply to soybean plants grown under shade. A previously described system was used to inject pressurized solutions into intact soybean plants. The experimental design was a split plot in which the main plot factor was shading level (0, 30, 50, and 70%), and the subplots were soybean plants injected with four different solutions, distilled water, 150 g sucrose L^{-1}, 15 mM nitrogen, and sucrose (150 g sucrose L^{-1}) plus nitrogen (15 mM). The average uptake during the injection period (8 weeks) was 66.5 mL. Plants injected with distilled water took up the most fluid followed by nitrogen, then nitrogen plus sucrose and finally sucrose solutions. The sucrose injected into soybean plants constituted an average of 47% of the total dry weight.

The amount of fluid taken up was not affected by shading. Under lower shading levels (0 and 30%), sucrose injection increased plant dry weight, with the highest dry matter accumulation being for plants under 0% shade and injected with sucrose. Under high levels of shading (50 and 70%), injection treatments did not increase plant dry matter, suggesting the existence of a light regulated mechanism that affects and possibly regulates the growth of shaded soybean plants.

7.1. Introduction:

For plant populations, competition is defined as two or more plants growing together in the same area and seeking the same growth factor, which is available at a level below their combined demand (Donald, 1963). Willey (1979) proposed that to improve the efficiency of multiple cropping production, competition between crops for growth limiting factors should be minimized. Francis (1987) noted that if water and nutrients are in sufficient supply, light would be the most limiting resource and that plants that are favored in an intercropping situation are those which are in best position to intercept light.

The reduced light environment encountered by shaded plants in multiple cropping systems constitutes a limitation to carbon assimilation. This is partly due to the limitation in the photosynthetic induction requirement that develops in this situation (Sassenrath-Cole and Pearcy, 1994). Egli (1988) reported that reducing light intensity tended to cause a reduction in the soluble sugar concentration in soybean plants. Regnier et al. (1988) noted an increase in the rate of photosynthesis per unit leaf volume of soybean plants under reduced irradiance. Burky and Wells (1991) found that maximum photosynthesis for soybean plants was decreased by two to threefold over a period of 40 days following a transition from a sun to a shade environment, and that maximum photosynthesis and chloroplast electron transport activity were stabilized or elevated in response to increased light intensity. At the whole plant level, shaded plants normally exhibit lower yields than their non-shaded counterparts. Wong and Stur (1996) found that shading forage grasses to levels as low as 20% caused a reduction in total dry matter yield. This decrease in yield was partly due to a decrease in the ability of the shaded plants to capture enough PAR to sustain optimum development. Shaded soybean plants have been reported respond to reduced light availability by an increase in the abscission of flowers and pods which eventually decrease yield (Jiang and Egli, 1993b).

Since a decrease in light intensity due to shading decreases the photosynthetic activity of shaded plants, the amount of carbon assimilated in these plants is reduced. Several studies have investigated the effect of adding an extraneous source of carbon, in the form of carbon dioxide, on the growth and development of soybean plants. Prior and Rogers (1995) reported that soybean plants exhibited increases in total leaf area, dry weight, and seed number when subjected to elevated levels of carbon dioxide. Reeves et al. (1994) reported a 34.7% seed yield increase in soybean placed under elevated levels of carbon dioxide. Allen et al. (1991) showed a seed yield increase of 20% in comparison with normal levels of carbon dioxide. Although carbon dioxide enrichment has been widely used as an extra source of carbon for greenhouse grown plants, plants tend to acclimate to such an increase in carbon dioxide enrichment through decreases in the ribulose bisphosphate carboxylase(Rubisco) content (Sassenrath-Cole and Pearcy 1994; XU et al. 1994) and stomatal opening (Fay and Knapp, 1995), leading, eventually, to photosynthesis levels similar to plants growing without carbon dioxide enrichment.

Low light levels have also been shown to decrease the ability of plants to fix nitrogen. Purcell and Sinclair (1993) reported that nodules of soybean plants placed under shade had a lower fractional air space content than those from unshaded plots; a lower fractional air space is associated with decreased nodule permeability to oxygen; an important determinant of nitrogen fixation.

Burkey and Wells (1991) showed that photosynthetic rates of soybean plants moved from a full-sun to a shaded environment were reduced by two to three fold over a period of 40 days. They also observed that light regulated both the photosynthesis and the timing of senescence in field-grown soybean leaves. Sassenrath-Cole and Pearcy (1994) reported that Rubisco deactivated very slowly in the dark and required higher light intensities for activation, however its activation was saturated at lower light intensities than photosynthesis. Babu and Nagarajan (1993) measured a decrease in the net photosynthesis of soybean plants placed under shaded conditions. Ikeda et al. (1993) reported that decreased irradiance caused a reduction in the amount of nitrate uptake by soybean plants, however its assimilation was not affected.

Gupta and Li (1994) showed that increased carbon dioxide levels reduced nitrogenase activity in the root nodule. Vidal et al. (1995) reported a decrease in acetylene reduction activity when soybean plants were exposed to carbon dioxide at compensation point levels, which they concluded was due to a decrease in the root nodule permeability to oxygen diffusion. Gupta et al. (1992) found that specific root nodule nitrogenase activity increased when soybean plants at the flowering stage were exposed to a combination of carbon dioxide and nitrogen dioxide. Hansen et al. (1992) reported a reduction in the nitrogenase activity of soybean when supplied with nitrates. Wu and Harper (1990) reported that the application of 5 mM nitrate resulted in greater inhibition of nitrogenase activity for soybean plants compared with controls. Fransisco et al. (1992) placed soybean plants under high irradiance and high soil nitrate levels and found higher irradiance was not enough to overcome the depressive effects of high nitrate on nodule activity, suggesting that carbon is preferentially used for nodule formation rather than nodule function.

Another possible form of carbon for supply to plants is sucrose. Sucrose has been successfully infused into barley plants a method developed by Ma and Smith (1992). Zhou and Smith (1996) developed a pressurized method and injected an average of 5.1 mL d^{-1} $plant^{-1}$ of concentrated sucrose solutions into corn. Abdin et al. (chapter 4) have modified the injection system developed by Zhou and Smith (1996) and were able to inject sucrose into soybean plants, at an average of 1.3 mL d^{-1} . Higher dry matter accumulation was recorded with plants injected with sucrose than injected with distilled water.

The objective of this experiment was to determine whether supplying soybean plants with extra sources of carbon and/or nitrogen in the form of sucrose and urea would mitigate the effects of shading.

7.2. Materials and Methods:

A greenhouse experiment was conducted in 1995 in the Plant Science Department of McGill University, Ste. Anne-de-Bellevue, QC, Canada. Seeds of the soybean cultivar "Maple Glen" were surface sterilized in sodium hypochlorite (2% solution containing 4 mL^{-1} Tween 20), then rinsed with distilled water several times (Bhuvaneswari et al., 1980). Seeds were planted in trays filled with 1:1 mixtures of sterilized sand and Turface (Applied Industrial Materials Corp., Illinois, USA). Seedlings were left to grow in the trays until they reached the VC stage [unifoliate leaves were unfolded sufficiently that the edges were not touching (Fehr and Caviness, 1977)]. Plants in the growing trays were watered as necessary. Vigorous seedlings were selected from the trays, and transplanted into 15.5 cm diameter and 15 cm deep plastic pots, containing the same rooting medium as the transplanting trays.

Prior to the experiment, 70 cm by 75 cm by 120 cm wooden frames were constructed out of 2.5 by 5.0 cm wood. The frames served as a support for the shading cloth that covered the entire box except the bottom side which was in contact with the greenhouse benches. Shading cloth (Tek Knit, QC, Canada) sufficient to provide 30, 50, and 70% light reduction were used to provide the shading necessary for each treatment. A 16-h photoperiod was maintained using supplemental lighting from high pressure sodium lamps. Temperature was maintained at 25 ±2°C. Relative humidity was approximately 75%.

After being transplanted into the pots, soybean plants were placed into the shading boxes and watered regularly using a modified Hoagland's solution (Hoagland and Arnon, 1950), in which $CaNO_3$ and KNO_3 were replaced with $CaCl_2$, K_2HPO_4, and KH_2PO_4 to provide a nitrogen-free solution. Soybean seedlings were inoculated with *Bradyrhizobium japonicum* strain 532C (Hume and Shelp, 1990). The inoculum was produced by culturing the *Bradyrhizobium* bacteria in yeast extract mannitol broth (Vincent, 1970) in 250 mL flasks shaken at 125 rpm at room temperature. Each plant received 2 mL of a 3-d-old (log phase) which was adjusted with distilled water to $O.D._{620}=0.08$ (approximately 10^8 cells mL^{-1}).

An injection system was established as described by Abdin et al. (chapter 6, Fig 6.1a) prior to the experiment. This was comprised of a supporting stand and a fluid injection system that terminated with a 25-gauge 3/4 vacutainer needle (Vacutainer, Becton Dickinson and Company, Rutherford, NJ). The vacutainer needle was bent to an angle of approximately 60° before insertion into the soybean stem. Soybean plants were injected with the vacutainer needle and the needles were sealed to the plant stems with latex (Vultex, General Latex Canada, Candiac, QC). The latex was placed around the injection site, in a cup formed of masking tape, and was allowed to set for a period of 7 days. Plants were tested for leaks and successful plants were placed under the shade ready for injection. Four plants were placed inside each shading box. Each plant was injected with a solution of either distilled water, 150 g L^{-1} sucrose, 15 mM nitrogen as urea, or 150 g L^{-1} sucrose plus 15 mM nitrogen. The injected solutions were forced inside the plants using ceramic bricks (approximately 2.7 kg each) placed on top of the syringe plunger. The bricks were a standard (22.5 cm by 8.5 cm by 7 cm) three hole construction type. One brick was added each day until reasonable flow rates were achieved. This never required more than 4 bricks.

The experimental design was a split plot factorial where the main factor was the shading treatment (0, 30, 50, and 70%), the subplot factor was injection solution [distilled water, 150g L^{-1} sucrose, 15 mM nitrogen, and sucrose (150g L^{-1}) plus nitrogen (15 mM)].

The amount of injected solution was monitored regularly, and the syringe barrels were refilled as necessary. The injected plants were examined daily to make sure there were no leaks. Plants were harvested at complete senescence (100% leaf drop). Abscised leaves were collected and weighed. Pods from each treatment were counted and oven dried to a constant weight at 90°C. Seeds were ground, and used to determine the grain nitrogen concentration by Kjeldahl analysis (Tecator, Kjeltec 1030 auto analyzer, Sweden). Plant height was measured from the soil surface to the tip of the stem. Nodules from each treatment were counted and dried to a constant weight at 90°C.

Nitrogen fixation was calculated by determining the total nitrogen in each plant, then subtracting from this value the amount of nitrogen contained in the seed from which the plant was grown and in the case of nitrogen injection plants, the amount of injected nitrogen. Days to regreening was used as an indication to the start of nitrogen fixation by soybean plants (Zhang et al., 1995)

Chlorophyll fluorescence measurements were recorded using a Morgan CF-1000 chlorophyll fluorescence measurement system (Morgan Scientific Inc., Andover, MA.). Three measurements were taken from each pot two weeks after the onset of injection. One cuvette per plant was placed on the uppermost fully expanded leaf. The cuvette was left for approximately 10 minutes on each leaflet so that the leaflets were acclimatized to darkness. The optical probe was then inserted into the cuvette and a reading was taken and used to determine the Fo (non-variable fluorescence), the Fm (maximal fluorescence), the Fv (variable fluorescence), and the ratio of Fv:Fm, which is a measure of the photochemical efficiency of photosystem II. This gives an indication of the stress status of the plants (Lichtenthaler, 1996). Readings from the 3 samples were averaged to calculate the soybean chlorophyll fluorescence for that treatment.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1985) was used for analysis of variance of all data. Probabilities equal to or less than 0.05 were considered significant for main effects and interactions. The least significant difference (LSD) test was used to separate differences between treatment means if analysis of variance indicated the presence of such differences (Steel and Torrie, 1980).

7.3. Results and Discussion:

7.3.1. Rate of infusion:

Insertion of the vacutainer needle did not injure soybean plants. During the first five days, the amounts of fluid absorbed were relatively small as pressure applied to the system was being increased slowly, by adding bricks, so as to avoid a sudden increase in pressure, which could rupture the system. Similar results were obtained by Abdin et al. (chapter 4) for soybean, and by Zhou and Smith (1996) for corn. After a period of seven weeks some blockage was seen in some of the treatments, mainly those containing sucrose. Those blockages were cleared by pulling the syringe plunger forward and backward several times.

Averaged over all the shading treatments, soybean plants injected with distilled water had the highest uptake rates, , followed by nitrogen, sucrose plus nitrogen and then sucrose solutions (Fig 7.2). A similar pattern was observed by Abdin et al. (chapter 6) for soybean and by Zhou and Smith (1996) for corn. The lower uptake rates for the more concentrated solutions were probably due to higher osmotic potentials. Ma et al. (1994) also noted a greater resistance of barley tissue to the infusion of concentrated sucrose solutions than water. The overall average uptake level was 66.5 mL (1.1 mL d^{-1}). Using a different, and more leak prone injection technique, Schon and Blevins (1987) managed to administer 18.5 mL $week^{-1}$ of relatively dilute amino acid solution to soybean plants.

Soybean plants receiving 150 g sucrose L^{-1} took up 8.1 g of the sugar, which represented 47% of the total dry weight of the plant (Table 7.4). Abdin et al. (chapters 6 and 8) were able to administer 11.8 g and 8.7 g representing 65 and 40% of the total plant dry weight in two different studies. Zhou and Smith (1996) reported an uptake of 17.7 g of sucrose when a 150 g L^{-1} sucrose solution was injected into corn plants. This was equivalent to 30% of the dry weight gain during the injection period.

7.3.2. Effects on soybean plants:

7.3.2.1. Shading:

Soybean plants growing under the heavily shaded treatments (50 and 70% shade) were taller than the unshaded or the lightly shaded (30%) plants which were not different from each other (Table 5.3). Plants under 30% or unshaded controls plants were taller when injected with sucrose, however under the 50 and 70% shade treatments, neither sucrose nor any other injected solution affected the height of soybean plants. Leaf number in the unshaded treatment was higher than those receiving shade treatments, and there were no differences among the shade treatments in the number of leaves (Table 7.1). The number of pods per plant was higher for unshaded control plants than any of the shaded treatments. Plants receiving 30% shade had higher pod numbers than those receiving 70% shade, but were not different from those under 50% shade (Table 7.1).

The number of grains per pod were not different among any of treatments with an average number of 1.6 grains per pod. The number of grains per plant was higher in plants receiving no shade than shaded plants, and plants receiving 30% shade had more grains per plant than those receiving either 50 or 70% shade, which were not different from each other (Table 7.1). The decrease in seed yield due to the shade treatments was mainly due to the lower number of pods produced. This is in agreement with results from Jiang and Egli (1993) who demonstrated a decrease in the number of soybean pods when subjected to shading and determined that the decrease was mainly due to increased flower and pod abscission.

The weight of unshaded soybean grains per plant was higher for unshaded plants than for those placed in shade, but there were no differences in grain weight among the shaded treatments (Table 7.2). Hayati et al. (1995) reported an increase in the dry weight of grains when shaded soybean plants were placed in an unshaded environment when compared with those maintained under shade.

Shoot dry weights for unshaded plants were higher than for those under shade (Table 7.2), presumably due to the better interception of photosynthetically active radiation, which in turn, leads to higher rates of carbon assimilation in the plants. Plants under 30% shade had higher shoot dry weights than those receiving 50 or 70% shading, and there was no difference between plants in the 50 and 70% shade treatments (Table 7.2).

Days to regreening (onset of nitrogen fixation, Zhang et al., 1995) in the 50% and 70% shading treatments was earlier by an average of 3-4 days compared with plants receiving either 30% or no shade. It appears that the development of all plant structures may have been accelerated by shade conditions. Shaded plants flowered sooner (visual observation) and senesced sooner (Table 7.3). As shaded plants developed smaller nodules they may have been able to reach complete formation sooner than the larger nodules formed on unshaded plants. Total nodule numbers and dry weights were higher in the unshaded treatment than any of the shaded treatments (Table 7.4).

Fluorescence values, Fv/Fm, for plants under the 70% shade were lower than those under no or 30% shade, but there were no differences between plants receiving either 50 or 70% shade, nor between unshaded plant and the 30% shaded plants (Table 7.3). It would appear that soybean plants were only stressed at elevated shade levels. This in addition to the larger leaves of the highly shaded plants (visual observation) indicating the production of "shade leaves", and an overall shift in the architecture and physiology of theses plants, could overshadow or possibly nullify any potential benefits from any of the injected solutions.

Root dry weight for the unshaded treatment was higher than those for any of the shaded treatments and plants receiving 50 and 70% shading had lower root weights than those plants placed under 30% shading (Table 7.2).

Total plant dry weight followed the same pattern as the shoot dry weight, being higher for unshaded plants than any of the shaded treatments, and plants receiving the 30% shading had higher dry weights than those receiving either 50 or 70% shading. There were no differences in the total dry weight for plants placed under either 50 or 70% (Table 7.2).

Shaded plants senesced approximately 14 days earlier than unshaded plants (Table 7.3). Plants in 30% shade senesced approximately 12 days earlier than the unshaded control. Pons and Pearcy (1994) showed that leaf senescence was enhanced by shading. Senescence of soybean plants was delayed by a period of more than four weeks when plants were in a less shaded environment (Burkey and Wells, 1991).

7.3.2.2. Injected solutions:

The injected solutions affected only the unshaded plants and those under 30% shade. The effects were less pronounced for the 30% shaded plants than the unshaded plants. Plants placed under either 50 or 70% shading did not show any effect of injection regardless of the solution being administered.

Unshaded and 30% shaded plants injected with sucrose had higher pod numbers than any of other plants under the same shading level (Table 7.1). Prior and Rogers (1995) reported an increase in the number of soybean pods when plants were placed under elevated levels of carbon dioxide. However, unshaded sucrose injected plants had higher pod numbers than those under the 30% shading treatments. There was no effect of the injected solutions on the total number of leaves.

For both unshaded and 30% shaded plants, seed dry weight of sucrose injected plants was higher than the rest of the injection treatments. They were followed by plants injected with sucrose plus nitrogen. However, plants receiving only nitrogen did not have significantly different seed dry weights than plants injected with distilled water.

Total plant dry weight of unshaded plants was higher for the sucrose injected treatment than any of the others, followed by those receiving sucrose plus nitrogen, which was not different from those receiving only nitrogen, and then plants receiving only water. The same pattern occurred for plants under 30% shading. The shoot dry weights responded to the applied treatments in the same way as total plant dry weight (Table 7.2).

Nodule weights under 0% and 30% shade treatments were higher for treatments receiving only sucrose and sucrose plus nitrogen, followed by those receiving water then plants injected with nitrogen. Root dry weight was increased by sucrose injection but only for unshaded plants.

Unshaded plants senesced later when injected with sucrose followed by plants injected with sucrose plus nitrogen, and then by plants injected with only nitrogen which senesced 3 days later than those receiving distilled water. A delay in senescence in response to injection was not observed in any of the other shade treatments.

Grain nitrogen concentrations in plants injected with nitrogen and sucrose plus nitrogen were higher than those injected with sucrose or water, which were not different from each other.

Nitrogen fixation was not affected by any of the injection treatments including those that contained nitrogen (Table 7.4). Shading did reduce plant nitrogen fixation. Plants unshaded or under 30% shade were not different from each other. This was probably because the more heavily shaded plants produced less dry matter and therefore had less nitrogen demand than the 30% shaded or unshaded plants. Injection of nitrogen lead to increased nitrogen concentration in tissues, as seen in seeds (Table 7.4) instead of reduction in nitrogen fixation. This extends the work of Cho and Harper (1991), who found that application of nitrogen fertilizer to one side of a legume split root system did not affect nitrogen fixation on the other side. In our study, the application of nitrogen to the stem did not inhibit nitrogen fixation in the roots.

7.4. Conclusions:

Plants at higher shade levels (50 and 70%) seem to have changed profoundly when compared with unshaded or 30% shaded plants. The more shaded plants were taller, senesced earlier and had lower fluorescence values, than the 30% or unshaded plants. The more shaded plants also failed to respond to the injected sucrose, while the unshaded and 30% shaded plants weighed more when injected with sucrose. These data suggest that both the physiology and architecture of the more shaded plants had undergone a clear shift in response to the shading, such that they developed and responded to the injected solutions quite differently from the unshaded or 30% shaded plants. The observation that injected nitrogen did not reduce nitrogen fixation provides further evidence that nitrogen fixation is not regulated by the overall level of nitrogen within a legume plant but rather by the local level of nitrogen detected by a given portion of legume roots.

Fig 7.1. Average weekly uptake of solutions during an 8 week period. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled water.

Fig 7.2. Total amount of injected solution during an 8 week injection period. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled water.

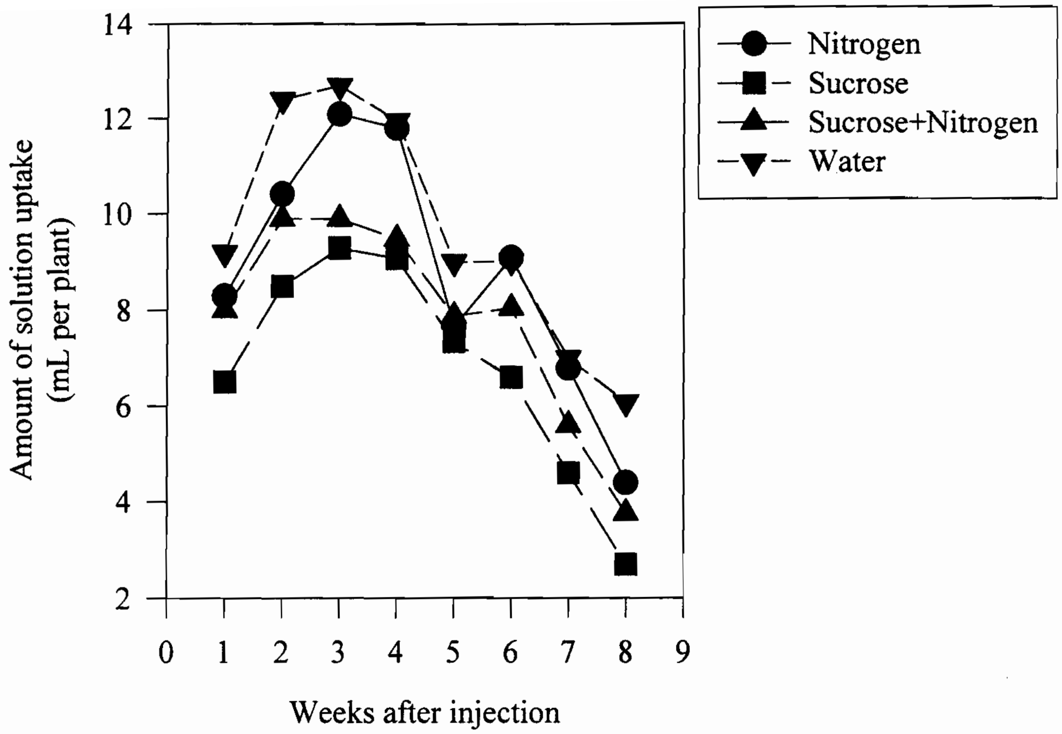

14
12
10
8
6
4
2
Amount of solution uptake
(mL per plant)
0
1
2
3
4
5
6
7
8
9
Weeks after injection
Nitrogen
Sucrose
Sucrose+Nitrogen
Water

Fig. 7.1.

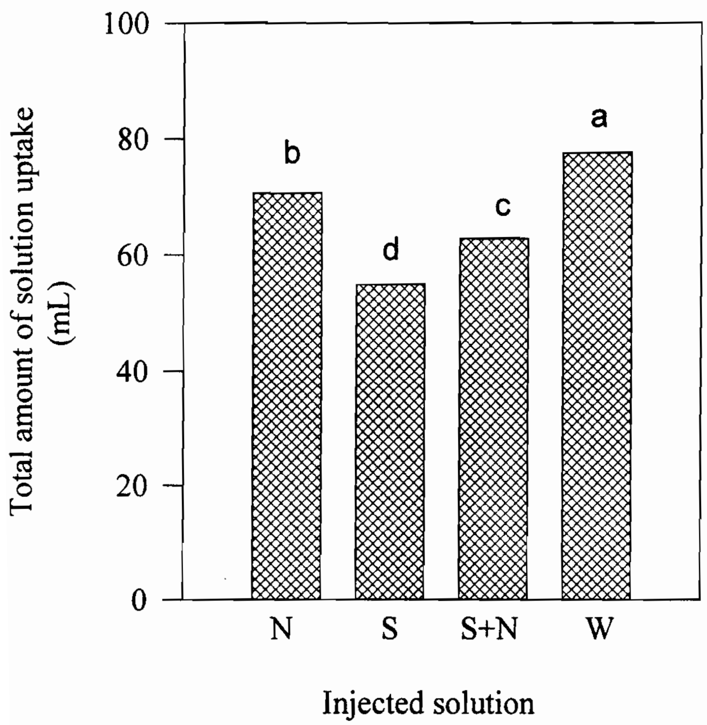

100
80
60
40
20
0
Total amount of solution uptake
(mL)
b
d
c
a
N
S
S+N
W
Injected solution

Fig. 7.2.

Table 7.1. Effects of the injected solutions under three levels of shading on aspects of soybean plant development.

Shade Level	Injected Solution	Plant Height	Leaf Number per Plant	Pod Number per Plant	Grain Number per Plant
0%	Nitrogen	62.5	52.7	19.7	31.1
	Sucrose	74.0	54.2	23.5	37.0
	Sucrose+Nitrogen	65.2	56.0	23.0	36.8
	Water	52.7	50.75	19.75	32.0
30%	Nitrogen	64.0	41.5	16.0	25.2
	Sucrose	72.7	45.5	18.0	29.7
	Sucrose+Nitrogen	65.0	48.0	15.0	24.7
	Water	50.7	47.0	15.5	24.0
50%	Nitrogen	133.7	39.0	12.7	20.0
	Sucrose	131.2	38.2	13.5	21.3
	Sucrose+Nitrogen	134.5	36.7	13.0	20.1
	Water	135.5	45.2	12.5	20.6
70%	Nitrogen	163.2	32.2	13.2	21.8
	Sucrose	164.2	34.5	13.2	21.8
	Sucrose+Nitrogen	162.0	38.2	13.2	20.7
	Water	164.2	45.5	12.2	19.4
LSDa		2.2	1.7	0.9	1.7
LSDb		2.1	1.4	0.6	1.4
LSDc		4.2	2.9	1.4	2.9

LSDa is for comparing means within the same whole plot level.
LSDb is for comparing means across whole plot factor levels.
LSDc is for comparing means with interaction effects. It is not included if the interaction did not occur

Table 7.2. Effects of the injected solution under three shading levels on elements of soybean plant weights.

Shade Level	Injected Solution	Shoot Weight	Grain Weight	Root Weight	Dry Weight	Weight per grain
		(g)	(g)	(g)	(g)	(g)
0%	Nitrogen	13.0	5.2	1.051	19.8	0.17
	Sucrose	16.0	6.6	1.125	24.3	0.18
	Sucrose+Nitrogen	13.0	5.9	1.015	20.3	0.16
	Water	10.1	4.9	0.925	16.6	0.15
30%	Nitrogen	11.5	4.3	0.900	17.1	0.17
	Sucrose	13.7	5.3	0.805	20.3	0.18
	Sucrose+Nitrogen	12.0	4.4	0.850	17.8	0.18
	Water	10.8	4.0	0.804	16.2	0.16
50%	Nitrogen	10.2	3.4	0.812	14.8	0.17
	Sucrose	11.0	3.8	0.819	16.0	0.17
	Sucrose+Nitrogen	11.1	3.4	0.825	16.0	0.17
	Water	12.0	3.7	0.658	16.9	0.18
70%	Nitrogen	10.0	3.5	0.772	14.7	0.16
	Sucrose	11.0	3.8	0.675	15.9	0.17
	Sucrose+Nitrogen	11.1	3.7	0.725	16.1	0.18
	Water	10.7	3.5	0.675	15.4	0.18
LSDa		0.5	0.2	0.07	0.5	0.006
LSDb		0.7	0.3	0.06	0.8	0.006
LSDc		1.4	0.6	----	1.6	0.012

LSDa is for comparing means within the same whole plot level.
LSDb is for comparing means across whole plot factor levels.
LSDc is for comparing means with interaction effects. It is not included if the interaction did not occur

Table 7.3. Effects of injected solutions under three shading levels on soybean plants.

Shade Level	Injected Solution	Fv/Fm	Days to Senescence
0%	Nitrogen	0.8013	119
	Sucrose	0.8222	121
	Sucrose+Nitrogen	0.8310	116
	Water	0.8101	116
30%	Nitrogen	0.8020	115
	Sucrose	0.8013	115
	Sucrose+Nitrogen	0.7998	113
	Water	0.8136	116
50%	Nitrogen	0.7452	105
	Sucrose	0.7345	106
	Sucrose+Nitrogen	0.7996	104
	Water	0.7532	106
70%	Nitrogen	0.7942	106
	Sucrose	0.7363	105
	Sucrose+Nitrogen	0.7823	107
	Water	0.7782	104
LSDa		0.0476	5
LSDb		0.0480	6
LSDc		---	10

LSDa is for comparing means within the same whole plot level.
LSDb is for comparing means across whole plot factor levels.
LSDc is for comparing means with interaction effects. It is not included if the interaction did not occur.

Table 7.4. Effect of different injection solutions under three shading levels on soybean plants.

Shade Level	Injected Solution	Seed Nitrogen (%)	Nodule Number	Nodule Weight (g)	Days to Regreening	Fixed Nitrogen (g)
0%	Nitrogen	6.9	126.2	0.480	21	0.65
	Sucrose	6.5	157.5	0.587	19	0.62
	Sucrose+Nitrogen	7.0	143.2	0.497	20	0.55
	Water	6.7	144.7	0.540	22	0.53
30%	Nitrogen	7.1	114.7	0.440	20	0.50
	Sucrose	6.7	130.2	0.447	20	0.51
	Sucrose+Nitrogen	7.0	139.0	0.512	21	0.50
	Water	6.6	136.0	0.545	20	0.52
50%	Nitrogen	7.0	121.7	0.422	17	0.47
	Sucrose	6.8	135.7	0.442	18	0.49
	Sucrose+Nitrogen	7.0	135.2	0.547	18	0.49
	Water	6.7	133.0	0.492	17	0.48
70%	Nitrogen	7.2	126.2	0.442	17	0.47
	Sucrose	6.7	134.0	0.407	17	0.49
	Sucrose+Nitrogen	6.9	135.5	0.555	18	0.48
	Water	6.5	136.2	0.477	18	0.47
LSDa		0.5	7.4	0.05	1.1	0.03
LSDb		0.3	6.4	0.03	1.0	0.02
LSDc		---	----	0.07	2.0	---

LSDa is for comparing means within the same whole plot level.
LSDb is for comparing means across whole plot factor levels.
LSDc is for comparing means with interaction effects. It is not included if the interaction did not occur.

Preface to Chapter 8

This chapter is part of a manuscript by Abdin et al. to be submitted to the Crop Science journal for publication in 1997. The format has been changed to conform to a consistent format within this thesis. All literature cited is listed at the end of the thesis. Each table or figure for chapter 8 is presented at the end of this chapter.

This chapter examined within plant competition for carbon and nitrogen resources which plays an important role in the senescence of leguminous plants. Using the injection technique developed through the work described in chapter 6, solutions containing sucrose and nitrogen and a combination of both were injected from the flowering stage until physiological maturity to determine their effects in reducing within-plant competition for resources.

Chapter 8

CARBON AND NITROGEN SUPPLEMENTATION TO SOYBEAN THROUGH STEM INJECTION AND ITS EFFECT ON SOYBEAN PLANT SENESCENCE.

8.0. Abstract:

Many plant senescence studies have noted that internal competition for nutrients such as carbon and nitrogen can be an important factor in the initiation of senescence. Recently, a technique was developed that allows the injection of large amounts of reduced carbon and nitrogen containing solutions into soybean plants over periods of weeks to months. Using this technique soybean plants were injected with solutions of sucrose (150 g L^{-1}), nitrogen (15 mM), sucrose plus nitrogen, and distilled water from the onset of flowering until senescence. The average uptake rate of all solutions was 1.2 mL d^{-1} per plant. Sucrose injected plants accumulated the most biomass, followed by those injected with sucrose plus nitrogen, then nitrogen, and distilled water injected plants. Soybean plants injected with sucrose senesced two weeks later than those injected with distilled water. Plants injected with nitrogen senesced three days later than those receiving only distilled water. Results demonstrate that nitrogen is not the limiting factor leading to the onset of senescence in soybean plants, but that reduced carbon plays an important role in this process.

8.1. Introduction:

Senescence has been described by Jiang et al. (1993a) as " a phase of leaf ontogeny marked by declining photosynthetic activity that, in soybean (*Glycine max* [L.] Merr.), is paralleled by a decline in chloroplast function". Several studies have been conducted to identify the mechanisms regulating senescence. For centuries farmers have known that by removing flowers and fruits of annual plants they were able to extend the longevity of these plants. This suggests that monocarpic senescence is controlled by processes within the plants that are linked to flowering and seed production (Leshem et al. 1986).

The first attempt to study the cause of senescence was made by Molisch (1938) in which he concluded that reproductive organs such as flowers and seeds withdraw nutrients from the rest of the plant to such an extent that little is left for the vital growth of the plant, resulting in senescence. Leopold et al. (1959) reported that soybean leaves maintained a green color until maturity when the flowers were removed.

Carbon limitation to the leaves has been shown to accelerate senescence. Burkey and Wells (1991) reported that senescence of soybean plants grown under field conditions was delayed by more than four weeks when plant populations and thus mutual shading by adjacent plants was reduced. Pons and Pearcy (1994) reported that leaf senescence of soybean plants was accelerated by shading when compared with plants growing under high light intensities. These studies imply that light regulated the timing of senescence through the supply of reduced carbon to the rest of the plant.

Sinclair and de Wit (1975) further elaborated the theory of within plant nutrient competition, by estimating the amount of photosynthate and nitrogen per hectare per day accumulated by several crops. Dividing the estimated amount of nitrogen uptake by the photosynthate production rate they derived an estimated 20 mg N g^{-1} of photosynthate during grain fill insufficient to satisfy the nitrogen demands of a crop such as soybean, which would require 29 mg g^{-1} of photosynthate. Therefore, the nitrogen demand by seeds was not totally satisfiable through nitrogen absorption from the soil or through fixation from the atmosphere. The extra amount would have to have been obtained from the vegetative plant parts. This eventual leads to the depletion of nitrogen and protein in the vegetative tissues, such that the plant loses its physiological activity through "self-destructive" removal of material to developing seeds. Such plants develop senescence during seed development, leading to a shortened period of seed development, and lowering the total potential yield. Sinclair and de Wit (1975) also hypothesized that the self destructive plants would be unresponsive to nitrogen fertilization during seed fill unless they increased their rates of nitrogen uptake during that period.

A recent study by Guiamet and Giannibelli (1996) reported a 90% loss in leaf soluble proteins by two soybean varieties in the period leading up to abscission, "stay-green" isolines of the two cultivars showed less protein degradation (50%) at the time of abscission. However, these mutants were not different from the original varieties in the breakdown of Ribulose bisphosphate caboxylase in leaf extracts.

Hayati et al. (1996) reported that only 17 mM N was required by soybean seedlings for maximum cotyledon growth and that media nitrogen levels had little effect on cotyledon dry matter accumulation in tissue culture, suggesting that soybean seeds can accumulate dry matter without accumulating nitrogen, and thereby not supporting the theory of enhanced senescence due to seed N demand. Hayati et al. (1995) reported that increasing photosynthesis by removing shade from shaded soybean plants at R5 did not accelerate leaf senescence, but it did increase the rate on nitrogen uptake. These results suggest senescence may be regulated by other processes in the leaves and not by seed nitrogen demand.

Plant hormones signals have also been shown to play a role in senescence. Nooden et al. (1984) removed pods from one branch of a two branched soybean plant and found that the depodded branch stayed green while the intact one senesced, suggesting that the origin of the senescence signal is the seed itself and not a nutrient supplied by the root system.

Recently, an injection technique has been adapted for soybean (Abdin et al. chapter 4). This allows injection of substantial amounts of a solution into soybean plants over a long period of time.

We have used this technique to further study the theory of within plant nutrient competition on the processes involved in the development of senescence in soybean plants. The objective of this study was to determine the effect of increased supplies of carbon or nitrogen on senescence of soybean plants.

8.3. Materials and Methods:

A greenhouse experiment was conducted in 1995 (Plant Science Department of McGill University, Ste. Anne de Bellevue, QC, Canada). Soybean (*Glycine max* [L.] Merr. cv 'Maple Glen') seeds were surface sterilized in sodium hypochlorite (2% solution containing 4 mL^{-1} Tween 20), then rinsed with distilled water for several times (Bhuvaneswari et al. 1980) and planted in trays filled with 1:1 mixtures of sterilized sand and Turface (Applied Industrial Materials Corp., Illinois, USA). Seedlings were left to grow in the trays until they reached the VC stage [unifoliate leaves were unfolded sufficiently that the edges were not touching (Fehr and Caviness, 1977)]. Plants growing in the trays were watered as necessary. Vigorous seedlings were selected from the trays, and transplanted into 15.5 cm diameter and 15 cm deep plastic pots, and containing the same rooting medium as the trays. After being transplanted into the pots, soybean plants were watered regularly with a modified Hoagland's solution (Hoagland and Arnon, 1950), in which $CaNO_3$ and KNO_3 were replaced with $CaCl_2$, K_2HPO_4, and KH_2PO_4 to provide a nitrogen-free solution. Soybean seedlings were inoculated with *Bradyrhizobium japonicum* strain 532C (Hume and Shelp, 1990). The innoculum was produced by culturing the *B. japonicum* cells in yeast extract mannitol broth (Vincent, 1970) in 250 mL flasks shaken at 125 rpm at room temperature. Each plant received 2 mL of a 3-d-old (log phase) culture which was adjusted with distilled water to O.D. $_{620}$=0.08 (approximately 10^8 cells mL^{-1}). A 16-h photoperiod was maintained using supplemental lighting from high pressure sodium lamps. Temperature was maintained at 25 ±3 °C. The relative humidity was approximately 75%.

An injection system was set up according to Abdin et al. (chapter 4) prior to the experiment. This was comprised of a supporting stand and a fluid injection system that terminated with a 25-gauge 3/4 vacutainer needle (Vacutainer, Becton Dickinson and Company, Rutherford, NJ). The vacutainer needle was bent to an angle of approximately 60° before insertion into soybean plant. Using masking tape, a triangular cup shape was formed around the stem of each soybean plant about 1 cm above the soil surface. Using the winged end of the injection tubing, the 25-gauge needle was inserted into the stem of each soybean plant, so that at least half the needle length was inside the stem. After needle insertion, the cup was filled with fluid latex (Vultex, General Latex Canada, Candiac, QC) and was left to dry for a period of 5 days, in order to ensure a proper seal at the injection site. The injection procedure was begun at the time of flower initiation. Due to the maturity of the plants, the stems were more lignified and needle insertion was more difficult than previous work (Abdin et al. chapter 6). We grew four times the number of plants needed for this experiment as the number of injection failures was high.

After drying the pots were placed on the injection stand, and the other end of the injection tubing was connected to a 5 mL-syringe. After withdrawing the air from the injection tubing, the syringe was then disconnected from the tubing, and filled completely (5 mL) with the treatment solution, placed in its designated place on the injection stand, and reconnected with the injection tubing. After assembling the injection system, pressure was applied to the syringe by placing ceramic bricks (approximately 2.7 kg each) on top of the syringe plunger. The bricks were a standard (22.5 by 8.5 by 7 cm) three hole construction type. One brick was added each day until reasonable flow rates were achieved. This never required more than four bricks. The amount of injected solution was monitored regularly, and the syringe barrels were refilled as necessary. The injected plants were examined daily to make sure that there were no leaks.

The experiment was organized in a randomized complete block design with four blocks. One block was established each week for four weeks providing replication in time. The treatments consisted of a distilled water injected control, 150 g sucrose L^{-1}, 15 mM N (as urea), and sucrose (150 g sucrose L^{-1}) plus nitrogen (15 mM).

Plants were harvested at complete senescence (at 100% leaf drop). Abscised leaves were collected and weighed. Pods from each treatment were counted and oven dried at 90°C to a constant weight. Plant height was measured from the soil surface to the tip of the stem. Nodules from each treatment were counted and dried in a 90°C oven until reaching a constant weight.

Chlorophyll fluorescence measurements were recorded using a Morgan CF-1000 chlorophyll fluorescence measurement system (Morgan Scientific Inc., Andover, MA, USA). Three measurements were taken from each pot. One cuvette per plant was placed on the uppermost fully expanded leaf. The cuvette was left for about 10 minutes on each leaflet so that the leaflets were acclimatized to darkness. The optical probe was then inserted into the cuvette and a reading was taken and used to determine Fo (non-variable fluorescence), the Fm (maximal fluorescence), Fv (variable fluorescence), and the ratio of Fv:Fm, which is a measure of the photochemical efficiency of photosystem II. This gives an indication of the stress status of the plants (Lichtenthaler 1996). Readings from the three samples were averaged to calculate the soybean chlorophyll fluorescence for that treatment.

The GLM procedure of the Statistical Analysis System (SAS Institute, 1985) was used for analysis of variance of all data. Probabilities equal to or less than 0.05 were considered significant for main effects and interactions. The least significant difference (LSD) test was used to separate differences between treatment means if analysis of variance indicated the presence of such differences (Steel and Torrie, 1980).

8.4. Results and Discussion:

8.4.1. Rate of infusion:

Plants that were successfully injected did not show any negative reaction to the injection procedure. During the first week, the amount of absorbed solution was always low. This was because the supplied pressure was increased gradually over this period so as to avoid any ruptures in the system. Similar results were obtained by Abdin et al. (chapter 6) for soybean, and by Zhou and Smith (1996) for corn.

The general pattern observed was that rates of solution injection were constant during the first four weeks of injection, after which the amount taken up declined for all solutions (Fig. 8.1). Sucrose was most affected by this decline, by week six plants were absorbing less than 1 mL day^{-1}, and by week eight absorption had stopped (Fig. 8.4). Other solutions followed the same pattern as sucrose solutions but declines were more gradual. Two reasons could have contributed to the reduced rates of sucrose uptake, first the higher osmotic potential of the sucrose solution and second, a build up of lignified and callus material in response to the injection wound. Fourtan-pour and Smith (1996) observed a decline in the uptake of solutions administered to barley plants where the needle tips rested in the tissue free space inside the peduncles of barley plants. Distilled water had the highest average uptake rate followed by nitrogen, then sucrose plus nitrogen, and lastly by the sucrose solution (Fig. 8.2). Similar results were reported by Zhou and Smith (1996) who found that corn plants had higher uptake rates for distilled water than a concentrated sucrose solution. Ma et al. (1994) also noted greater resistance of barley tissues to the entry of concentrated sucrose solutions and related this to the high osmotic potential of these solutions.

Adding nitrogen to the sucrose solution facilitated the uptake of the sucrose (150 g L^{-1}) by the soybean plants compared with plants injected with only sucrose. The overall average uptake was 71.2 mL (1.2 mL d^{-1}). Abdin et al. (chapter 4) reported similar values when soybean injection was administered for 8 weeks starting at the VC stage. Using a different technique, Schonn and Blevins (1986) were able to administer 18.5 mL $week^{-1}$ of amino acid solution into soybean plants using a technique developed by Grabau et al. (1986). Grabau et al. (1986) indicated that this technique could be effective but was leak prone. It would probably not have been applicable with concentrated sucrose solutions as the pressures required would have caused constant leakage.

Plants receiving sucrose took up an average of 8.7 g of sucrose, representing 40% of the total dry weight of the soybean plants receiving the 150 g sucrose L^{-1} solution. Abdin et al. (chapter 6) managed to administered 11.8 g of sucrose to soybean plants that were injected at the VC stage. This represented 65% of the total dry weight of the resulting soybean plants. Zhou and Smith (1996) reported an uptake of 17.7 g of sucrose through injection of a 150 g L^{-1} sucrose solution by injected corn plants. Boyle et al. (1991) were able to infuse approximately 15 g of sucrose per corn plant. Zhou and Smith (1996) reported the addition of sucrose equivalent to 30% of the dry matter gain during the injection period for corn plants.

8.4.2. Effect on soybean plants:

Leaf number of soybean plants was not affected by any of the treatments in this study. However, leaf weights for plants injected with sucrose were higher than those receiving any of the other treatments, which were not different from each other (Fig. 8.3). This could be attributed to the increase in reduced carbon supply available to the sucrose injected soybean plants. Similarly, Reinert and Ho (1995) found that soybean plants subjected to elevated levels of carbon (in the form of carbon dioxide) had higher leaf weights than control plants.

The number of pods on soybean plants receiving only sucrose or those receiving sucrose plus nitrogen, were higher than those receiving only distilled water. However, pod number for plants receiving only nitrogen were not different from the control treatment

(Table 8.4). Prior and Rogers (1995) reported an increase in the number of soybean pods per plant when plants were subjected to elevated levels of carbon dioxide. Hayati et al. (1995) reported an increase in the dry weight of seeds produced per plant in response to removal of shade from shaded plants and that this result was enhanced by the presence of added nitrogen in the soil. Pod dry weight responded to the injection treatment in the same way as for pod number, except that pod dry weight for the sucrose injected plants was higher than any of the other treatments including the sucrose plus nitrogen treatment, which was in turn higher than those receiving only nitrogen or water (Fig 8.5). The average pod dry weights for plants receiving nitrogen only was not different from those receiving only water. Rogers et al. (1983) reported an increase in the seed yield of soybean plants subjected to elevated levels of carbon dioxide due to a greater seed number per plant.

Shoot dry weights for soybean plants receiving sucrose were greater than those injected with sucrose plus nitrogen. The shoot dry weights of the sucrose plus nitrogen injected plants were higher than those of plants receiving only nitrogen or only distilled water. The dry weights of the sucrose plus nitrogen injected plants may have been lower than those of the sucrose injected plants, because of a combined osmotic and salt stress effect. Ma and Smith (1994a) found that injection of nitrogen containing compounds at concentrations above 15 mM caused some symptoms of salt stress in barley, a salt tolerant crop. Zhou et al. (unpublished data) found that injection of sucrose concentrations above 150 g L^{-1} was stressful to corn plants. The injection of either 150 g sucrose L^{-1} or 15 mM nitrogen L^{-1} into soybean plants did not produce any stress symptoms. The plants grew normally and appeared healthy. However, it may be that injection of a solution containing 150 g sucrose L^{-1} plus 15 mM nitrogen L^{-1} was stressful, leading to the observed decrease in dry matter production relative to sucrose-only injected plants. This situation may have been exacerbated by the fact that inclusion of the nitrogen solution facilitated plant uptake of the sucrose plus nitrogen solution relative to the sucrose alone (Fig 8.2).

There was no effect of any treatment on the number of nodules produced during the growth period. However, nodule weights were higher for the sucrose injected plants than the control treatments (Fig 8.8), indicating larger nodule sizes of sucrose injected plants. This is in agreement with finding of Abdin et al. (chapter 6) who found that the size of nodules on soybean plants injected with sucrose starting at the VC stage was higher than the control (distilled water injected) treatment. Root weight for the sucrose injected plants was higher than any of the other treatments, which were not different from each other.

Total dry weight for plants injected with sucrose was higher than any of the other treatments (Table 8.10). Similar results were reported by Prior and Rogers (1995), where plants had higher total dry weights when subjected to elevated levels of carbon dioxide. Sucrose injected plants were taller than those receiving only nitrogen, while there were no differences in plant heights among any of the other treatments (Fig. 8.11).

Sucrose injected plants senesced two weeks later than controls. Sucrose plus nitrogen plants senesced 10 days later than controls, and the time of senescence for the nitrogen injected plants was not different from those of the distilled water injected controls. This is in agreement with findings of Abdin et al. (chapter 7) unpublished data where soybean plants grown under shade senesced 2 weeks earlier than those without shade. Similar results were reported by Pons and Pearcy (1994) who found that soybean leaf senescence was enhanced by shading. Burkey and Wells (1991) reported a senescence delay of more than 4 weeks in plots where soybean populations were reduced so that shading by adjacent plants was reduced.

The delayed senescence of the sucrose injected plants in our experiment may have been due to the availability of an extra source of reduced carbon to soybean plants, which led to a delay in the decline in the photosynthetic activity of soybean plants.

8.5. Conclusions:

Sucrose injection contributed approximately 40% of the total dry weight of the sucrose injected plants. Dry weights of soybean plants injected with sucrose were higher than any of the other treatments. Sucrose injected plants senesced two weeks later than the distilled water injected plants. Sucrose plus nitrogen injected plants senesced 10 days after the distilled water control, and plants injected with nitrogen only senesced only 3 days later than the distilled water injected plants.

Fig 8.1. Average weekly uptake of solutions during an 8 week period. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

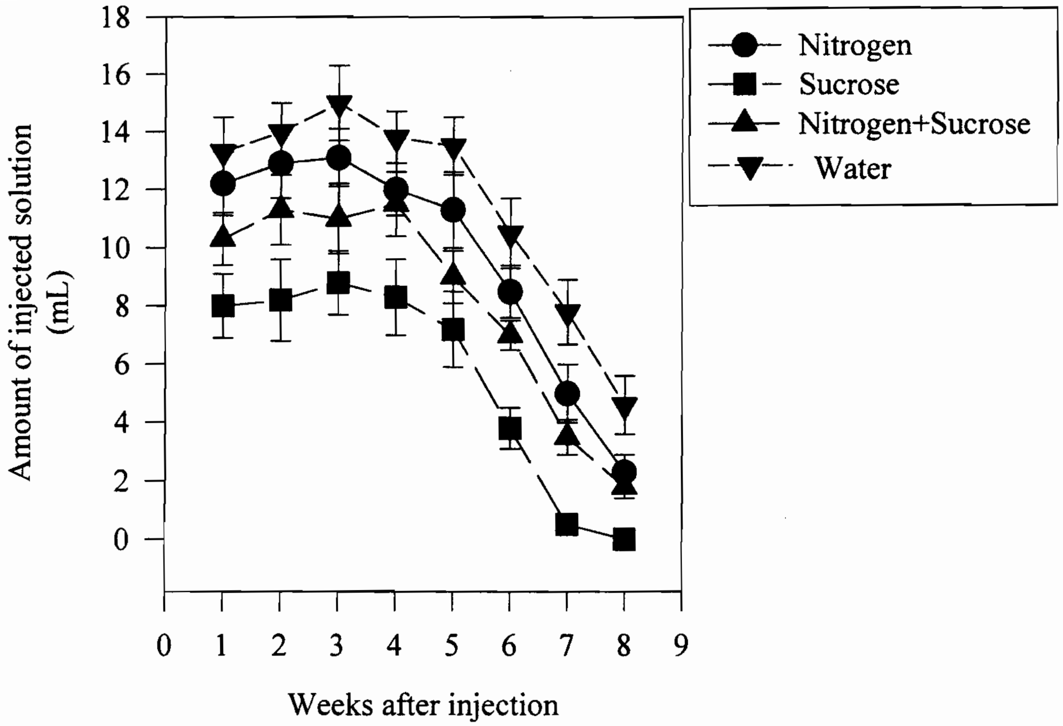
Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

Fig 8.2. Total amount of injected solution during an 8 week injection period. (the same letters indicate there were no differences between treatments at a 0.05 probability level).

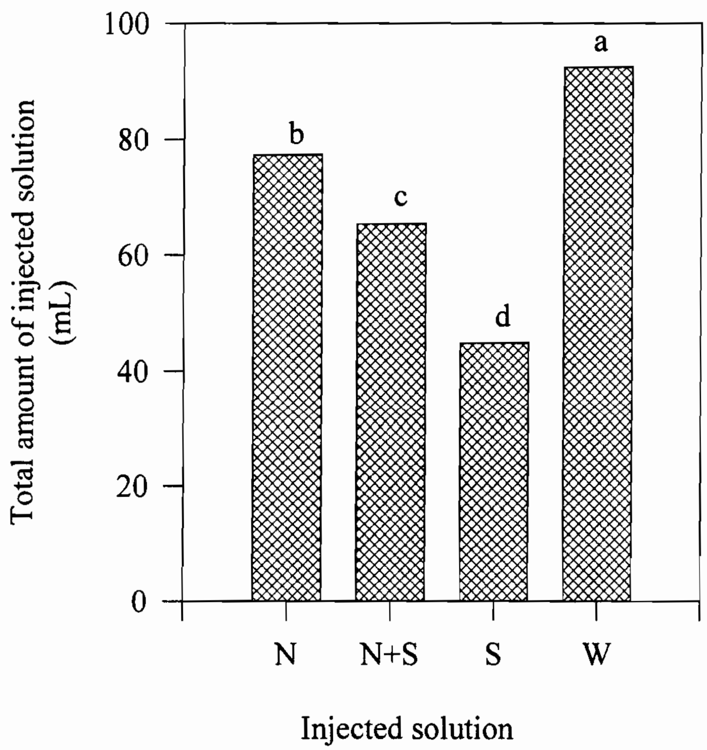
Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.



Amount of injected solution (mL)
Weeks after injection
Nitrogen
Sucrose
Nitrogen+Sucrose
Water
0
2
4
6
8
10
12
14
16
18
0 1 2 3 4 5 6 7 8 9

Fig. 8.1



Total amount of injected solution (mL)
Injected solution
0
20
40
60
80
100
b
c
d
a
N
N+S
S
W

Fig. 8.2

Fig 8.3. Effect of injection solution on soybean leaf weights. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

Fig 8.4. Effect of injection solution on the number of pods on soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

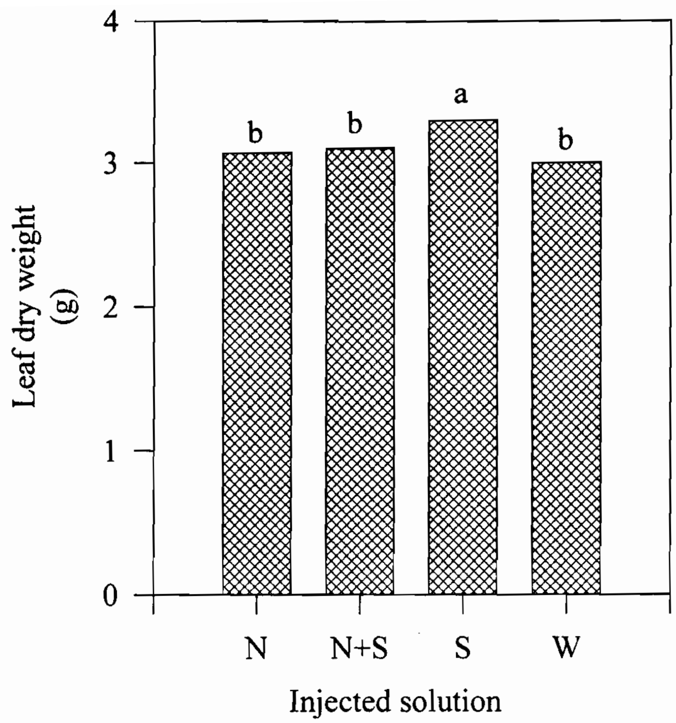

4
3
2
1
0
Leaf dry weight
(g)
b
b
a
b
N
N+S
S
W
Injected solution

Fig. 8.3

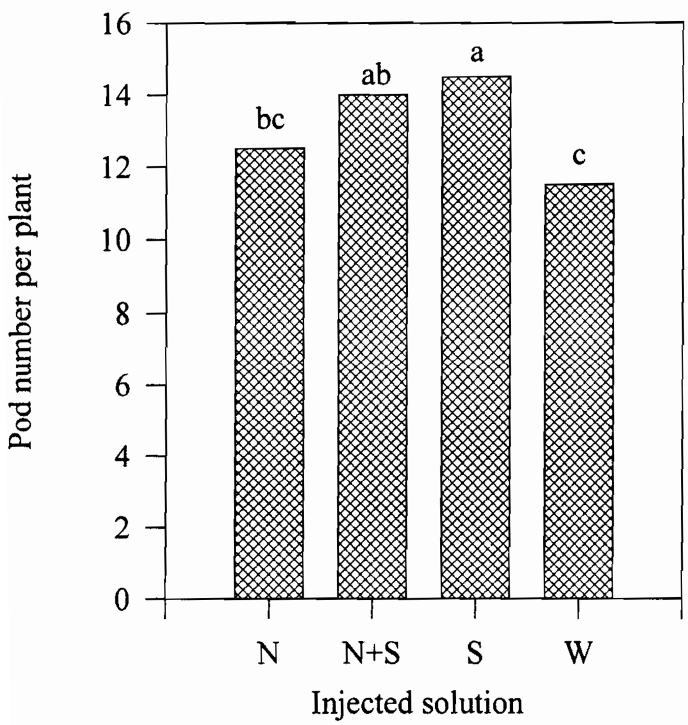

16
14
12
10
8
6
4
2
0
Pod number per plant
bc
ab
a
c
N
N+S
S
W
Injected solution

Fig. 8.4

Fig 8.5. Effect of injected solution on the pod weight of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

Fig 8.6. Effect of injected solution on shoot weight of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

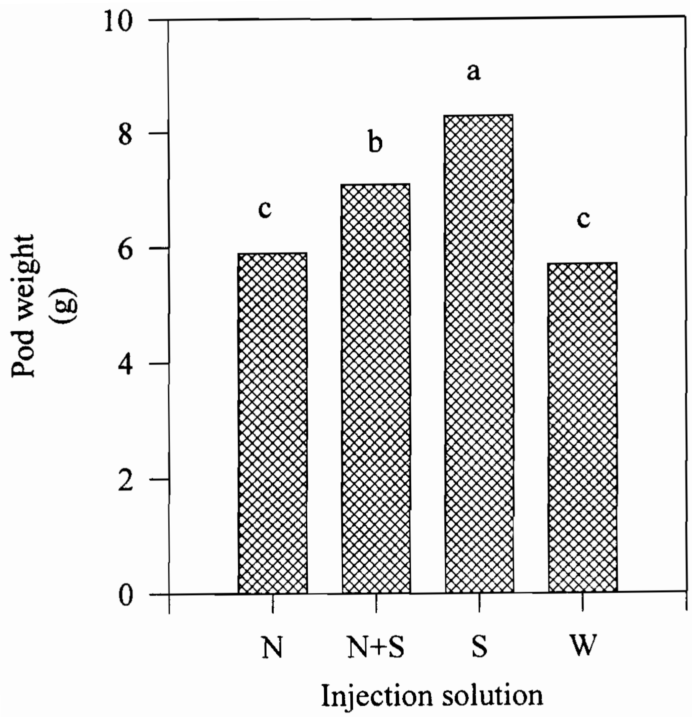

10
8
6
4
2
0
Pod weight
(g)
a
b
c
c
N
N+S
S
W
Injection solution

Fig.8.5

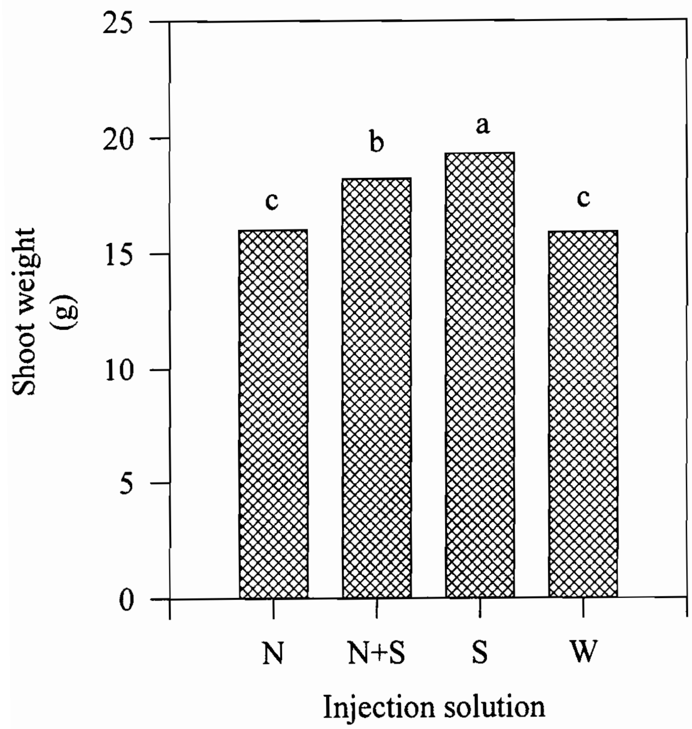

25
20
15
10
5
0
Shoot weight
(g)
a
b
c
c
N
N+S
S
W
Injection solution

Fig. 8.6

Fig 8.7. Effect of injection solution on nodule number of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

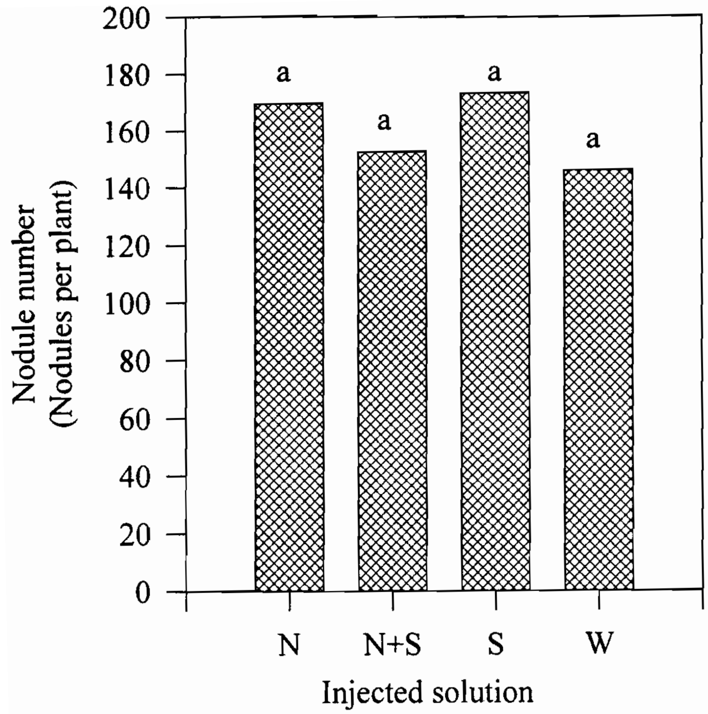
Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

Fig 8.8. Effect of injection solution on nodule weight of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

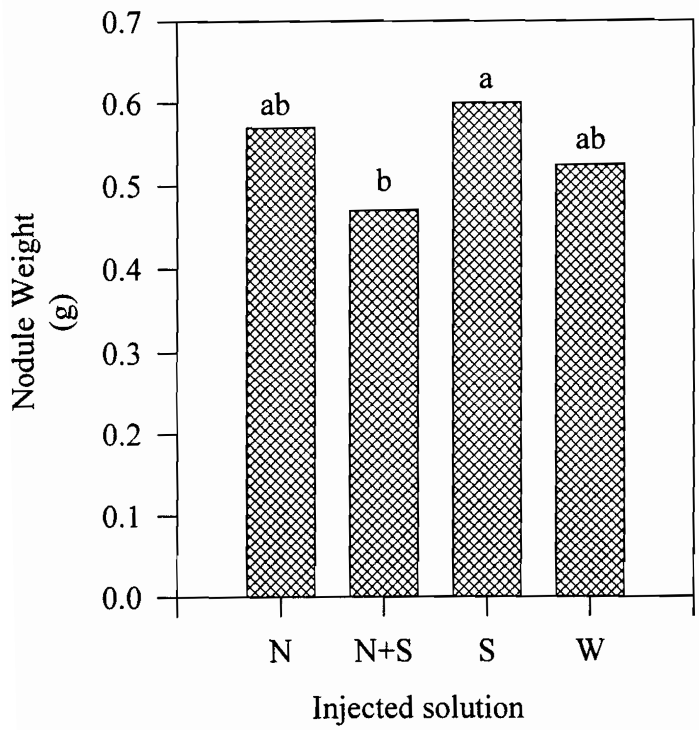
Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.



200
180
160
140
120
100
80
60
40
20
0
Nodule number
(Nodules per plant)
a
a
a
a
N
N+S
S
W
Injected solution

Fig. 8.7



0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Nodule Weight
(g)
ab
b
a
ab
N
N+S
S
W
Injected solution

Fig. 8.8

Fig 8.9. Effect of injection solution on root weight of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

Fig 8.10. Effect of injection solution on the total dry weight of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

Legend:

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

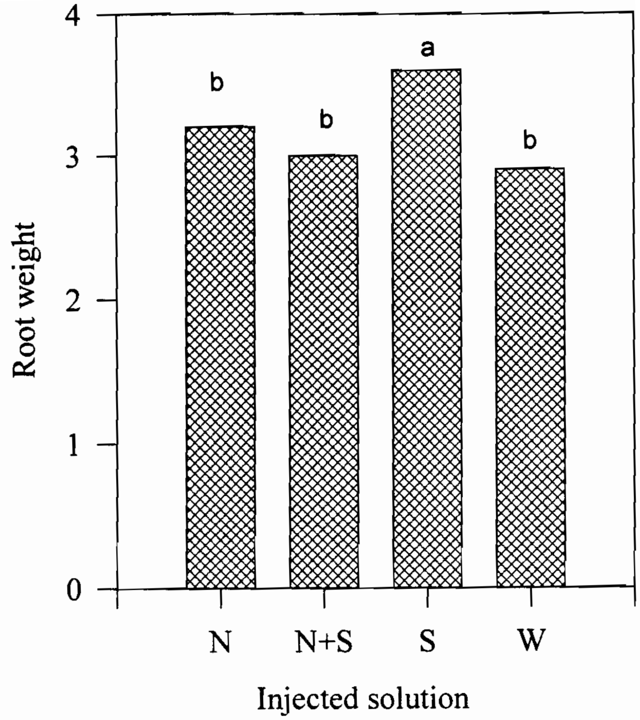


Fig. 8.9

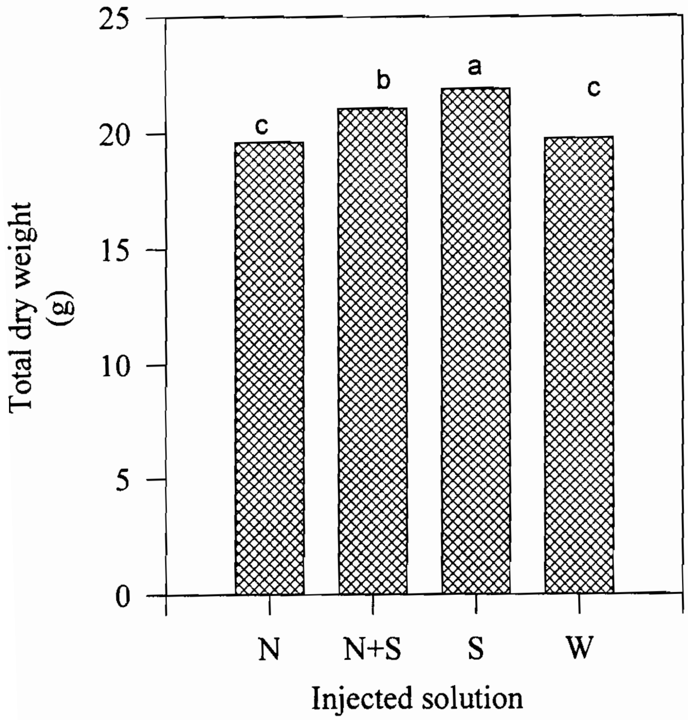


Fig. 8.10

Fig 8.11. Effect of injection solution on height of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

<u>Legend:</u>

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

Fig 8.12. Effect of injection solution on the senescence of soybean plants. (The same letters indicate there were no differences between treatments at a 0.05 probability level).

<u>Legend:</u>

N : 15 mM Nitrogen.
N+S : Nitrogen 15 mM plus sucrose 150 g L^{-1}.
S : Sucrose 150 g L^{-1}.
W : Distilled Water.

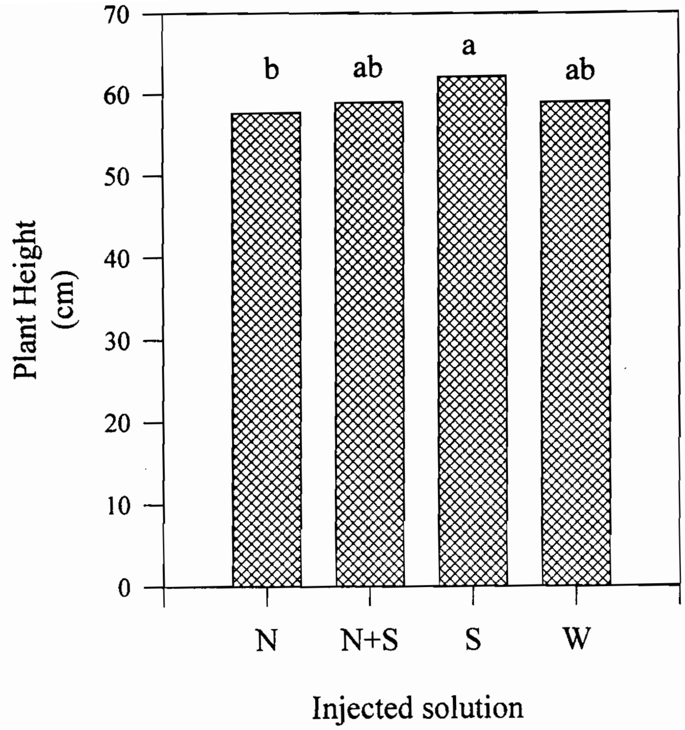


Fig. 8.11

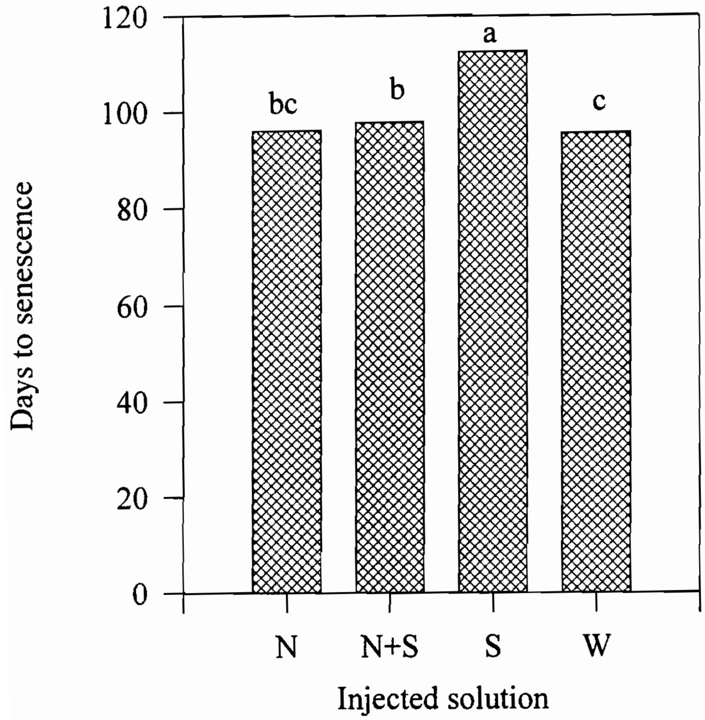


Fig. 8.12

Chapter 9

GENERAL DISCUSSION

In the field studies carried out in 1993 and 1994, corn was subject to more severe weed competition at the l'Assomption site than at the Macdonald site. The major weeds at l'Assomption were quack grass, common lamb's quarter, and foxtails. The lesser weed competition at the Macdonald site also allowed better establishment and biomass production by forages than at l'Assomption.

The time of interseeding of the forage legumes and grasses did not affect corn yields, nor did it enhance weed suppression. However, mean forage biomass was approximately 25% higher in earlier seeded treatments than late seeded ones. This was due to production of larger plants as there were no differences in population densities between seeding dates. Stute and Posner (1993) reported similar results, where forage legumes interseeded in corn were had lower dry matter yields when seeded later in the season. Early seeded forages were able to provide better early ground cover than late seeded ones, however this did not lead to greater reductions in weed populations. Strawberry clover provided poor ground cover earlier in the season. Fall rye and crimson clover provided the best soil cover of the interseeded cover crops. Despite the early ground cover provided by fall rye it achieved lower biomasses yield than crimson clover, due to relatively early senescence during the period leading up to corn harvest. Hairy vetch did not provide adequate ground cover early in the season, however higher biomass was attained later in the season, mainly due to the vertical growth habit of hairy vetch, which tends to grow over the corn stems rather than horizontally over the soil surface.

The results suggest that an earlier seeding date would be possible for interseeded forages under our conditions, which would allow better establishment for the forages and better suppression of weeds, with minimal or no reduction in corn yields. Several studies have indicated that the earlier planting of the interseed crops causes substantial reductions in corn grain yields (Schaller and Larson 1955; Ampong, 1985; Stemann et al., 1993), however, forage seeding as early as 10 days after corn emergence appears to be feasible in our environment. Scott and Burt (1985) recommended that species such as alfalfa, hairy vetch, and red clover be seeded when corn is 15 to 43 cm high. At 10 days after corn emergence our corn plants were approximately 14 cm high.

In 1994 crimson clover was among the highest biomass producing legumes, however, corn grain yields from plots interseeded with crimson clover were lower than for any other cover crop treatments, apparently due to the its early germination and better development.

Subterranean clover produced comparatively little biomass and provided low levels of ground cover. This could be attributed to the growth habit of subterranean clover, as it grows close to the soil surface without providing enough elevated leaf area to compete for light with weeds. These results contradict findings by Enache and Ilnicki (1990) who reported that subterranean clover provided good weed control.

During a wetter of the two seasons (1994) interseeding cover crops did not affect corn yields, however there were some reductions in a drier season (1993), indicating that production by the dominant crop can be very sensitive to water availability in polyculture systems. This, in combination with an additional cultivation prior to seeding the cover crops in 1994 may have contributed to the better 1994 corn grain yields. Similar results were obtained by Zhou et al. (personal communication) who found that intercropping corn with ryegrass did not reduce corn yields when sufficient water and nitrogen were available.

Results from the two years of this study suggest that yield increases in plots seeded the previous year with cover crops appeared to responsible for about a 0.5% increase in yield. While this may be a very slight increase, it may be noteworthy after only one year. Results by Decker et al. (1994) showed an increase in corn grain yield after cover crop legumes, seeded in the previous fall, as compared to after no cover crop.

Under severe competition from weeds and forage plants, corn plants could not manage complete compensation for lower cob numbers and yields were reduced (chapter 3). The various cover crop treatments had little effect on the number of kernels per cob, suggesting that none of the treatments affected corn plants at the seed setting stage, and further suggesting that any yield reduction effects occurred at a later stage, during grain filling.

In general the presence of weeds in the weedy control decreased corn yields except for the wet season of 1994 (chapter 4). There was a negative correlation between weed biomass for plots interseeded with crimson clover, and berseem clover, however the lower grain yields obtained from these plots was due to competition from both weeds and well established forages, but mainly due to the forages as the weed biomass in these plots was generally lower than the other the treatments (chapter 4). In general decreases in weed biomass were accompanied by a higher forage biomasses in plots seeded with red clover plus ryegrass, crimson clover, and berseem clover (chapter 5).

Lack of differences between treatments for the concentration of grain protein argues against meaningful levels of nitrogen transfer from any of the forage legumes to associated corn plants.

Cultivation prior to seeding cover crops proved to be very effective in reducing weed populations later in the season. The choice of cultivator has also been shown to be an important factor in reducing weed populations. In 1993 higher weed biomasses were observed on the corn rows than to between the corn rows. This was due to the fact that in 1993 cultivator tines were not placed on the rows, so as to avoid corn damage, where as in 1994 the tines were placed in a downward position so as to cultivate the area near the corn rows more vigorously which lead to a lower on row weed biomass than 1993, thereby contributing to better corn yields than the previous year.

In 1994 the combination of cover crops and interrow tillage controlled 80 and 75% of the weeds at the Macdonald and l'Assomption sites, respectively. Cultivation alone controlled 70 and 80% of the weeds present at the Macdonald and l'Assomption sites respectively. Thus the cover crops alone were responsible for only about 10% of the weed control at Macdonald. However this was not true for the highly infested l'Assomption site, indicating some effectiveness for using cover crops in fields with lower weed populations but not for very weedy ones. In very weed infested fields herbicide treatment would be more appropriate than interseeding cover crops.

Except for 1993 at the l'Assomption site berseem clover had greater dry matter yields than alfalfa, indicating the greater suitability of berseem clover than alfalfa for use in interseeding systems. However, in the presence of high weed populations berseem clover failed to establish and was out yielded by alfalfa.

In order to study more closely the effect of low light levels experienced by the cover crops and how they relate to the carbon and nitrogen nutrition of the plant an injection system was developed to administer exogenous solutions containing carbon and nitrogen to plants. Soybean was chosen as a model plant because it is a legume, it has relatively thick stems that would make injection easier, and it is a widely grown field crop. The system developed was successful in injecting substantial amounts of dilute and concentrated solutions of sucrose and nitrogen into soybean plants. The soybean plants absorbed less of the solutions with higher osmotic potentials, but this was at least partly overcome by the application of more pressure (bricks) to force the solution into the plants. Uptake of the injected solution was more rapid during the first 3 to 4 weeks of injection. After that period a decline in the amount of injected solutions was observed, regardless of the solution being administered. This decline was probably due to the build up of callus material around the injection site, which would have impeded the uptake of the injected solutions by partially obstructing the opening of the injection needle. Despite lower intake rates at the end of the injection period, the injected solutions affected the growth and development of injected soybean plants. The average overall solution uptake across experiments was approximately 75 mL $plant^{-1}$ during an eight week injection period. The quantities of sucrose injected constituted 60-65% of the total dry weight of the plants.

The injection technique allowed a substantial amount of reduced carbon to be injected into the plants. The amounts injected were larger than has been possible with previous methods such as sugar uptake by leaf absorption.

When plants were injected at the onset of flowering it was more difficult to establish an injection site, due to the higher levels of lignified material in the soybean stem than was than was the case for the VC stage plants used in earlier work. The injection of nitrogen alone beginning at flowering did not affect the grain yield of the soybean plants, however, injecting sucrose plus nitrogen or sucrose alone increased the number and weight of soybean pods. Rogers et al. (1983) reported an increase in the seed yield of soybean plants subjected to elevated levels of carbon dioxide, and Hayati et al. (1995) reported similar results, and also added that the increases in seed weights were enhanced by the presence of higher levels of nitrogen in the soil. Injection of soybean plants with sucrose plus nitrogen resulted in delayed senescence relative to those injected with nitrogen alone or distilled water. This delay was largely due to the sugar component of the injected solutions.

Sinclair and de Wit (1975) suggested that plants such as soybean are forced into senescence due to depletion of nitrogen within the plant tissues, and that the duration of seed development is tied up closely with the rate of nitrogen uptake. They also suggested that unless the plants could increase nitrogen uptake during seed filling, nitrogen demands by the seeds will exceed that which is absorbed by the plant roots and this will necessarily result in lower seed yields than would otherwise be the case. However, our results show that despite higher uptake rates for nitrogen during seed fill period, due to the injection of

N containing solutions, N injected plants senesced only slightly later than hose injected only with distilled water. Another recent study by Hayati et al. (1995) disagreed with the Sinclair and de Wit theory in that they reported that leaf senescence does not occur because of seed nitrogen demand, but may be regulated by processes in the leaves themselves.

Soybean plants under heavily shading conditions senesced two weeks earlier compared to those under unshaded conditions (chapter 7). The early senescence of interseeded fall rye in the field (chapter 5) may well have been a similar shade acceleration of senescence. As this acceleration senescence occurred in the absence of any reproductive development it would not have been related to competition between reproductive and vegetative structures.

Chapter 10

CONTRIBUTIONS TO KNOWLEDGE

1. Twelve forage legumes and grasses, and two grass legume mixture were evaluated as interseeded cover crops for corn. This is the first extensive evaluation of a wide range of cover crops to be reported for this region.
2. Some cover crops that have been shown to work well as cover crops further south (e.g. hairy vetch) do nott work well here.
3. Cover crops can provide weed control in fields with moderate weed populations but not in fields with high populations.
4. Seeding cover under corn had little or no effect on corn grain yield
5. Cover crops can be seeded closer to the corn seeding date than is the case further south.
6. Soybean plants were able to uptake substantial amounts of concentrated water soluble solutions through stem injection. This is the first research that reports the successful development of an injection technique whereby concentrated solutions could be injected under pressure into soybean plants.
7. Supplying exogenous sucrose and nitrogen to shaded soybean plants did not affect highly shaded plants, but did affect unshaded plants. This is the first time the effects of increases in the supply of sucrose and nitrogen has been investigated under shaded conditions using an injection technique.

8. Carbon and nitrogen injection delayed senescence of soybean plants. This work has not been previously possible due to the limitations in the ability to introduce carbon, other than carbon dioxide, to soybean plants, and due to the regulation of nitrogen uptake by the plant.

Chapter 11

SUGGESTIONS FOR FUTURE RESEARCH

The following work would extend the findings of this thesis:

1. A field trial testing an even earlier seeding date for the cover crops under the environmental conditions prevailing in eastern Canada.
2. Further study regarding seeding rates and seeding dates for crimson clover when produced as a promising cover crop under corn.
3. Evaluate selected cover crops and tillage for weed control in the newly available leafy-reduced stature corn.
4. Investigate other methods for increasing carbon supply for soybean plants under shading conditions, such as elevating carbon dioxide supply, to allow a better understanding of within plant competition for reduced carbon.
5. With the success of the injection technique other solutions such as plant hormones could be injected to soybean to determine their role in whole plant senescence or processes such as nodulation.

Chapter 12

ACCEPTANCE OR REJECTION OF HYPOTHESIS

Hypothesis 1:

Corn yield is not affected by late interseeding of forage legumes and grasses between corn rows.

Results related to hypothesis 1:

In this study there were no effects of the time of interseeding forage legumes and grasses on the grain yield of corn. Thus, **we accept hypothesis 1 but note that early seeding is also workable management strategy.**

Hypothesis 2:

Different cover crops have diferent abilities to provide adequate cover under corn canopies.

Results related to hypothesis 2:

Our results show that fall rye and crimson clover provide good ground cover and establish well under corn canopies, however strawberry clover and hairy vetch did not provide adequate cover nor did they establish well under corn canopies. Thus, **we reject hypothesis 2.**

Hypothesis 3:

Interseeded cover crops will suppress weed populations in grain corn.

Results related to hypothesis 3:

Some of the interseeded forages such as crimson clover and the mixture of white clover and ryegrass were able to suppress weed populations. However other cover crops were not able to do so. Thus, **we accept the hypothesis but only for selected treatments.**

Hypothesis 4:

Cultivation in combination with cover crops provides an efficient means of controlling weed populations.

Results related to hypothesis 4:

Cultivation accounted for approximately 70% of the reduction in weed population, which proves that it was mandatory for the successful establishment of the cover crops and better weed control. Thus, **we accept hypothesis 4.**

Hypothesis 5:

Soybean plants can be injected with water soluble solutions throughout the bulk of their growth and development.

Results related to hypothesis 5:

Soybean plants were able to uptake up to approximately 75 mL of pressurized solutions during an eight week injection period. Thus, **we accept hypothesis 5**.

Hypothesis 6:

Growing soybean plants under shade simulates the shading aspect field conditions in an intercrop system and providing these shaded plants with sucrose and nitrogen, via stem injection, alleviates the detrimental effects of shading.

Results related to hypothesis 6:

Our results showed no effects of the injected sucrose or nitrogen for more heavily shaded soybean plants, however unshaded and slightly shaded plants were affected by the injection of these solutions. Thus, **we reject hypothesis 6**.

Hypothesis 7:

Injection of soybean plants with sucrose and nitrogen solutions throughout their productive period will reduce intra plant competition, resulting in a protracted reproductive period (delayed senescence) and greater yields.

Results related to hypothesis 7:

Soybean plants injected with sucrose senesced approximately two weeks later than control plants injected with distilled water. Plants injected with sucrose plus nitrogen senesced 10 days later than plants injected with distilled water, and plants injected with nitrogen only senesced 3 days later than distilled water injected controls. Thus, **we accept hypothesis 7.**

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